Characterization of Sediments and Mussels to Determine if Oil and Metal Contamination is Affecting the Scallop Population in Port au Port Bay, NL

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Abstract

This study investigated whether the decline of the scallop fishery in Port au Port Bay, Newfoundland was caused by hydrocarbon contamination. Potential hydrocarbon contaminants and sediments were chemically characterized for their organic and inorganic components. A new method for extracting sediment samples using accelerated solvent extraction was developed and applied to extract polycyclic aromatic hydrocarbons and alkanes from sediment samples. Water samples were chemically characterized for signs of inorganic and organic contamination. Since there were no scallops present at the study sites, mussels were used as a proxy organism. Mussels were analyzed for contaminants, $\Delta^{14}C$, and their health indices. No signs of contamination in the sediments, water, or mussels were detected. This data suggested the decline of the scallop fishery in Port au Port Bay cannot be explained by petroleum hydrocarbons from the leaking oil well.

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List of Abbreviations and Symbols

DFO- Department of Fisheries and Oceans
LNAPL - light non-aqueous phase liquids
PAH – polycyclic aromatic hydrocarbon
Pg – petagram
TIC – total inorganic carbon
DOC – dissolved organic carbon
$\Delta^{14}C$ – change in ^{14}C concentration
‰ - part per thousand
GC-MSD - gas chromatograph equipped with a mass spectrometer detector
ASE – Accelerated Solvent Extraction
PBFG – Port au Port Bay Fishing Grounds
PBSP – Port au Port Bay Shoal Point
PBFW – Port au Port Bay Fishing Wharf
PBSB – Port au Port Bay Sand Bar
GB – St. George's Bay
GBSB – St. George's Bay Sand Bar
GBSC – St. George's Bay Stephenville Crossing
pH – measure of hydrogen ion concentration
DO – dissolved oxygen
BDH – British Drug Houses
MCE – mixed cellulose ester
GF/F – glass microfiber filter
VOA – volatile organic analyte
DE – diatomaceous earth
DCM – dichloromethane
WAF – water-associated fraction
TLE – total lipid extract
NOSAMS - National Ocean Sciences Accelerator Mass Spectrometer
$\delta^{13}C$ – isotopic signature, a measure of the ratio of stable isotopes ^{13}C: ^{12}C, reported in parts per thousand
EPA – Environmental Protection Agency
RSD – relative standard deviation expressed as percent (%)
ICP-MS – Inductively Coupled Plasma Mass Spectrometer
ICP-OES – Inductively Coupled Plasma Optical Emission Spectrometry
d.f. – degrees of freedom
f - fraction
K_{ow} – the octanol/water partition coefficient
UNEP – United Nations Environment Programme
CCME – Canadian Council of Ministers of the Environment

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Chapter 1 Introduction

1.1 Study Significance

In autumn of 2012 scallop harvesters in Port au Port Bay, Newfoundland began noticing a decline in the number of scallops in the bay. By 2013 almost all of the scallops caught were empty shells (also known as "clappers") (Gale, 2014). This is the first time in recent history, that scallop harvesters have noticed such a drastic decline in the number of scallops present in the bay (Gale, 2014). In 2013 three scallop tows yielded 200 scallops; however, of these 200 all but 16 were "clappers" (Hillier, 2014). In past years, only approximately 20% of these 200 scallops would have been clappers (O'Gorman, 2014). The scallop fishing industry represents an important source of income for the residents of the Port au Port Bay area and it is estimated that the loss of this industry could cost fish harvesters between 25-30% of their total annual income (Hillier, 2014). In 2013, the Department of Fisheries and Oceans (DFO), Newfoundland and Labrador, visited Port au Port Bay and collected live scallops from the area and tested them for diseases. The tests determined the scallops were free of any diseases and therefore, the cause of the decline was still unknown. The scallops, however, were not tested for metal or organic contamination. Conversely, the adjacent bay, St. George's Bay, was reported to not be experiencing the same problem with their scallop population (Hillier, 2014). St. George's Bay is located south of Port au Port Bay on the western coast of Newfoundland (Figure 1.1). The two bays are very close together. At its closest point, St. George's Bay is separated from Port au Port Bay by an approximately 300 m wide piece of land. Therefore, due to the similar geographical locations, St. George's Bay would serve as a

good comparison site to help identify the cause of the decline of the scallop population in Port au Port Bay.

Figure 1.1 A Google Earth image showing study sites on the west coast of Newfoundland: Port au Port Bay and St. George's Bay (Google Earth 7.1.5.1557: May 28, 2012). Port au Port Bay, NL Canada. 48°27'04"N, 58°15'35.35"W, Eye alt 50.61 km. SIO, NOAA, U.S. Navy, NGA, GEBCO. TerraMetrics 2015. <http://www.google.ca/earth/index.html> (Accessed March 11, 2015).

There could be many potential factors contributing to the decrease in scallop abundance such as parasite infection, overfishing, increasing sea temperatures, acidification, or contamination (CBC, 2012; Garcia, 2006; Jonasson et al., 2007). The goal of this thesis was to determine if the scallop fishing grounds in Port au Port Bay were being impacted by organic and/or inorganic contaminants. St. George's Bay was

used for comparison as it was geographically close to Port au Port Bay and its scallop population has not experienced the same decline as Port au Port's (Hillier, 2014). This research will help determine if a point source of contamination was impacting the scallop fishery in Port au Port Bay. Environmental policies should be put in place, if the point source is impacting the bay, to prevent further damage to the delicate marine ecosystem on the west coast. Additionally, environmental remediation strategies should be implemented to preserve Port au Port Bay.

1.2 Potential Sources of Organic Contamination and Health Impacts on Marine Biota

There appear to be two potential sources of organic input in the Port au Port Bay area. The first potential source of organic input is crude oil either from natural seeps and/or leaking from drilled oil exploration wells, both of which exist in and around Port au Port Bay. Natural seeps of oil have been reported along the western coast of Newfoundland for more than 200 years (Hicks and Owens, 2014). Alexander Murray described a natural oil seep at Shoal Point that was later confirmed by James Howley in 1874 when he visited the site (Hicks and Owens, 2014). These seeps can still be seen at Shoal Point when holes are dug into the beach (Figure 1.2).

Bitumen and oil stained rocks are also known to occur in Port au Port Bay and hydrocarbons have been reported in drilled water wells since the 1940's in West Bay (Hicks and Owens, 2014). This is because the Port au Port region is located on the Green Point shale. This shale is part of an allochthon (part of the Earth's crust that has been

moved from its point of origin) and is a potential host to shale oil and shale gas (Hinchey et al., 2015). The Green Point shale is heavily fractured, crisscrossing the rock layers at various angles. These fractures in the formation result in the leaking of hydrocarbons and explain the abundant seeps visible throughout the Port au Port region (Hinchey et al., 2015).

Figure 1.2 Oil seep near Shoal Point on the Port au Port Peninsula (CBC, 2015).

Exploratory oil drilling in the Port au Port region began as early as 1890 when BHP Petroleum Limited drilled four wells at Shoal Point (Hicks and Owens, 2014). Since then oil exploration has been ongoing periodically; however, reports indicate that a

minimum of 13 oil wells were drilled at Shoal Point alone (Figure 1.3) (Hicks and Owens, 2014).

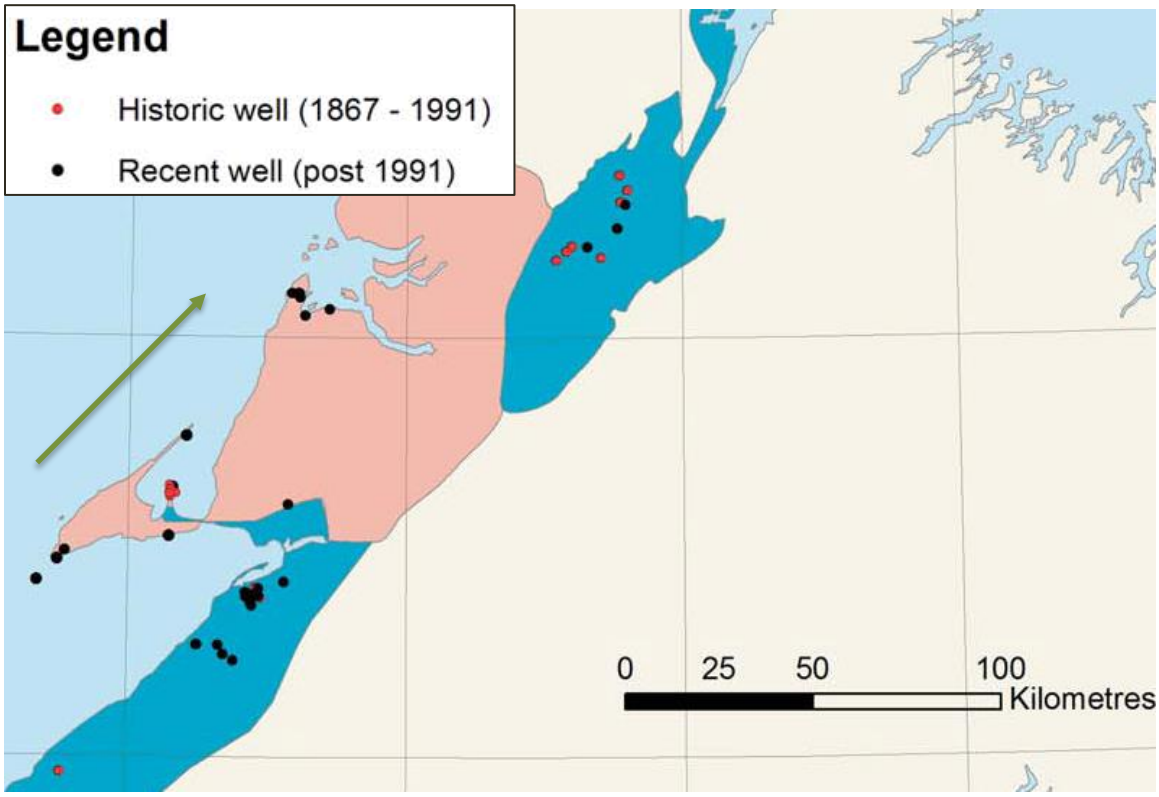

Figure 1.3 Oil wells in western Newfoundland. Historic wells are indicated in red and recent wells are indicated in black. The pink zones represent lower Paleozoic Basins and the blue zones represent upper Paleozoic Basins (Hicks and Owens, 2014). The green arrow represents the mean water current direction in the area (Environment Canada).

In 2013 residents of the Port au Port Bay area identified a number of oil wells that were in the bay (i.e. submersed in water) due to coastal erosion (Figure 1.4). These wells were leaking an oily substance and were potential hydrocarbon point sources.

Figure 1.4 Oil well in Port au Port Bay submerged due to coastal erosion (Gale, 2014).

The second potential source of organic contamination in the area could be refined petroleum and diesel used in boat engines. Fishing (i.e. scallop, lobster, and crab) is an important source of income for many families on the west coast of Newfoundland. Fish harvesters in the area rely on both commercial fishing vessels and smaller personal watercraft. The engines of watercraft can be powered by either diesel or gasoline and therefore, leaky engines, small fuel spills, or oil slicks could all be contributing to organic contamination in the bay.

Oil spills can pose a large threat to marine ecosystems: even small-scale spills can have detrimental impacts on an environment (Hannam et al., 2010b). Two of the largest marine oil spills in recent memory include the Exxon Valdez and the Deepwater Horizon

oil spills. The Exxon Valdez oil spill, which occurred in March 1989, released 40 million liters of oil impacting approximately 2100 kilometers of shoreline (Neff *et al.* 1995). The spill affected many organisms and only now, over two decades later, oil from the spill has disappeared from all but a small portion of the shoreline (Wiens, 2013). In April 2010, the largest environmental disaster in the history of the United States occurred: the BP Deepwater Horizon oil leak in the Gulf of Mexico (Barron, 2012). It is estimated that approximately 780 million litres of crude oil were released into the water column (Atlas and Hazen, 2011). Over 1,600 km of shoreline were affected by this spill and over 20 million hectares were closed to fishing. Many species of birds, mammals, fish, and reptiles were, and continue to be, affected by this spill (Barron, 2012).

When released into an aquatic system oil spills pose a risk to the environment through different mechanisms. Light non-aqueous phase liquids (LNAPL) pose a risk through direct discharge from the point source (EPA, 1995). Additionally, constituents of the LNAPL that are soluble in water (i.e. benzene, xylene, and toluene) can dissolve and produce plumes. These plumes are more mobile than the LNAPL and therefore, are often harder to contain and remediate (EPA, 1995).

The toxicity of oil spills to an environment is largely due to the stability of certain compounds (i.e. polycyclic aromatic hydrocarbons) in oil and their resistance to degradation in seawater and sediments (Blummer et al., 1970). If a spill is not properly contained and cleaned it can have significant impacts on marine biota over a large area. Additionally, after the initial spill has dispersed, oil can still continue to seep from the sediments exposing benthic organisms to elevated concentrations of polycyclic aromatic

hydrocarbons (PAHs) and alkanes (two major components of crude oil) (Hannam et al., 2010b).

After the Exxon Valdez oil spill, post-spill clean-up efforts removed most of the oil in intertidal areas of Prince William Sound with high-pressure hot water (Carls et al., 2001). Unfortunately, due to the damage this method could cause to delicate environments, mussel beds were unable to be cleaned using this method (Carls et al., 2001). In 2001, Carls et al. found that six years after the initial spill, sediments in mussel bed areas still remained contaminated with petroleum hydrocarbons. Mussels living on these sediments were also found to contain petroleum hydrocarbons in their biomass and experienced a reduced fitness and lower air survival rate than those not exposed to oil. Mussels containing petroleum hydrocarbons also posed a significant toxic threat to predatory species in the area through trophic transfer of contaminants (Carls et al., 2001).

Bivalves are a class of sedentary filter feeding organisms that include scallops and mussels. They have a tendency to accumulate organic compounds in their body tissues through passive diffusion where they undergo biotransformation reactions producing reactive oxygen species (Hannam et al., 2010a; Hannam et al., 2010b). Studies have suggested that exposure to these compounds, primarily PAHs, can reduce the function of the immune system of bivalves (Geraldine McCormick-Ray, 1987; Hannam et al., 2010b). Immune defense is largely regulated by blood cells that recognize foreign material and destroy it either through ingestion or secretion. PAHs can impair this cellular response, thereby compromising the immune system of bivalves (Hannam et al., 2010b). A study published in 2002 found that bivalves collected from an area impacted by the Exxon Valdex oil spill, a decade after the initial spill, showed signs of cellular

physiological stress when compared to bivalves from an unoiled area (Downs et al., 2002). For this reason many studies have relied on the use of scallops and mussels as indicators of water quality in a marine environment (Burns and Smith, 1981). For example, a study conducted in 1981 by Burns and Smith used the marine mussel *Mytilus edulis* as an indicator species of water quality. The authors were able to detect low levels of petroleum contamination in Victorian coastal waters in Australia. Their study suggested that *Mytulis edulis* quantitatively reflected the level of contamination they were exposed to in the water column. Hence, mussels may be a good indicator of petroleum contamination (Burns and Smith, 1981). Sampling the mussel population of Port au Port and St. George's Bays may be necessary if no live scallops can be collected.

1.3 Potential Sources of Metal Contamination and Health Impacts on Organisms

Heavy metals, metallic elements that have a density five times greater than that of water, are widespread throughout the environment through anthropogenic and natural activities. Many heavy metals are part of the Earth's crust and therefore, can be widespread due to physical weathering of rocks, soil formation, and volcanic eruptions (Bradl, 2005). However, most environmental contamination of heavy metals occurs through anthropogenic activities such as mining and smelting operations, coal burning, and petroleum combustion (Bradl, 2005). There appear to be two anthropogenic potential inputs of metal contamination into the Port au Port Bay: through crude oil contamination and a garbage dump identified by residents of the area. Studies have shown that some crude oils can contain small amounts of heavy metals (Fahim et al., 2010). Since heavy

metals are non-volatile, even when oil evaporates the heavy metal contamination can remain. Examples of heavy metals often found in crude oil include vanadium, nickel, iron, and copper (Fahim et al., 2010; Wang et al., 2009).

The presence of heavy metals present in landfills is largely due to their industrial uses including cadmium and lead in batteries, and chromium in paint pigments (Wang et al., 2009). When there is an excessive amount of rainfall in an area, landfill leachate is generated through the percolation of water through the layers of the landfill (Kjeldsen et al., 2002). Depending on the mobility of the heavy metal, which is largely determined by the speciation of the metal, it can enter the water column. This can cause exposure of hazardous metals to nearby environments and could have a detrimental impact on aquatic environments (Kjeldsen et al., 2002).

Heavy metals can have toxic effects even at very low levels of exposure (Wang et al., 2009). Cadmium, lead, and mercury are known as the most hazardous toxic heavy metals to humans and the environment. For many heavy metals, such as copper, there is a narrow range of concentration between beneficial and toxic effects. Diets that contain high levels of copper can lead to toxicity (Luckey et al., 1975). Other heavy metals such as cadmium, lead, and mercury have no established biological function (Wang et al., 2009). Methylmercury present in aquatic systems can be taken up by aquatic biotia and bioconcentrated. Bioconcentration factors as high as 10^5 to 10^7 have been reported meaning that accumulation in an aquatic food chain can be very high even when there are very low environmental conditions (Canada, 2013). Predatory aquatic wildlife species, organisms at the top of the food chain, are therefore exposed to the highest levels of

mercury and can experience negative impacts such as reduced reproduction rates and neurological effects (Bradl, 2005; Canada, 2013).

Unlike many organic pollutants, metals do not degrade to carbon dioxide and water in the environment (Wang et al., 2009). Heavy metals tend to accumulate in the environment, particularly in sediments. Due to the significant impact that heavy metals can have on the environment, we chose to study sampled water, sediments, and mussels for metal content.

1.4 Background

In aquatic systems, the carbon reservoir can be divided into inorganic carbon and organic carbon. Inorganic carbon is carbon present in its oxidized form; the sum of all the total dissolved carbon dioxide (i.e. including all inorganic species of carbonic acid, bicarbonate, and carbonate ion) is referred to as total inorganic carbon (TIC). Organic carbon is carbon present in its reduced form; it can be further subdivided into dissolved organic carbon (carbon that can pass through a 0.45 μm filter, referred to as DOC) and particulate organic carbon (carbon which remains on the 0.45 μm filter). In the ocean there is an exchange between the two pools of carbon; inorganic, for example can be converted to organic carbon through photosynthesis by certain primary producers, such as phytoplankton. Organic carbon can also be converted to inorganic carbon through respiration of non-photosynthetic organisms, for example zooplankton (Williams and Follows, 2011). Not all the organic carbon, however, will be converted back into inorganic carbon and a small fraction will reach the sea floor. Over time, this buried organic carbon can be converted into fossil fuels (Williams and Follows, 2011). In

aquatic systems, for example Port au Port Bay, fossil fuels can dissolve in the water phase. This dissolved fossil fuel is referred to as plumes and the carbon from these plumes contributes to the total DOC of the ocean.

Fossil fuels (crude oil) can be extracted from a reservoir. From there it is refined into petroleum products such as fuel for transportation (i.e. boats) and heating. This can be achieved through a variety of refining processes, for example distillation (Fahim et al., 2010). During distillation, the crude oil components are separated by boiling points through a series of heat exchangers. The crude oil is heated so that when it enters the atmospheric distillation column it is in vapour form. From there the vapour is transferred to a column where it will condense back to liquids that have been separated by weight (Fahim et al., 2010). Gasoline has a lower carbon chain range (typically around 6 carbon atoms) and therefore a lower density, while diesel has a heavier carbon range (typically between 14 to 20 carbon atoms) and therefore, a higher density (Fahim et al., 2010).

Conversely, unprocessed oil, crude oil, is a complex mixture of hydrocarbons, organic compounds, and metals. However, these hydrocarbons can be grouped into three main classes: saturated hydrocarbons, unsaturated hydrocarbons, and aromatic hydrocarbons. Alkanes, acyclic saturated hydrocarbons, are important constituents of crude oil. Polycyclic aromatic hydrocarbons (PAHs) are an example of aromatic hydrocarbons that contain two or more fused aromatic rings (Fahim et al., 2010). PAHs are an environmentally important constituent of oil; therefore, they have been used to determine if oil is impacting an environment (Wiens, 2013). The EPA has identified 16 PAHs that are on the priority pollutant list (Fig. 1.4). By analyzing samples for these PAHs and the ratio between the PAHs, chemical fingerprints can be created. PAHs of

molecular mass 178 and 202 are commonly used to determine if a compound is a product of combustion or petroleum. For example, a ratio of anthracene to anthracene plus phenanthrene of less than 0.10 generally indicates a petroleum based source, while a ratio greater than 0.10 suggests a combustion source (Yunker et al., 2002). Examples of PAH ratios observed in petroleum samples are shown in Table 1.1.

Table 1.1 Literature PAH ratios for petroleum (taken from (Yunker et al., 2002)).

Source	**BaA/∑228**	**IP/(IP + Bghi)**	**An/178**	**Fl/(Fl + Py)**
Crude Oil	0.12±0.06	0.09	0.07	0.22±0.07
Kerosene	0.35	0.48	0.04	0.46
Diesel	0.35±0.24	0.40±0.18	0.09±0.05	0.26±0.16

An/178 signifies ratio of anthracence to anthracene plus phenanthrene
Fl/(Fl +Py) signifies ratio of fluoranthene to fluoranthene plus pyrene
BaA/∑228 signifies ratio of benz[*a*]anthracene to benz[*a*]anthracene plus chrysene/triphenylene
IP/(IP + Bghi) signifies ratio of indeno[1,2,3-*cd*]pyrene to indeno[1,2,3-*cd*]pyrene plus benzo[*ghi*]perylene

Chemical fingerprinting has been applied to this study. Organic profiling of potential sources of contaminants in Port au Port (i.e. crude oil, gasoline, and diesel) can be compared to the organic profiling of the fishing ground sediment of the bay to determine the source of the organic contamination.

While both PAHs and alkanes have anthropogenic sources, for example the burning of fossil fuels, they also both have natural sources. Natural PAHs can result from forest fires, natural losses or seepage of petroleum or coal deposits, and volcanic eruptions ((CCME), 2008). Natural sources of alkanes include insect pheromones,

microbial biosynthesis, and plant cuticular waxes (Samuels et al., 2008; Schirmer et al., 2010; Tillman et al., 1999). Since both types of compounds commonly assessed in petroleum hydrocarbons can have anthropogenic and natural sources it is difficult using only the oil profiling approach to determine if their presence in organisms is through natural or anthropogenic sources. Additionally, distinguishing many of these organic compounds can be difficult because many organic compounds are often degraded or biotransformed in organisms (Morrill et al., 2014a). Therefore, molecular-level ^{14}C was used to identify the ancient carbon associated with petroleum in mussel tissue. Molecular-level ^{14}C, a technique based on the geological age difference between petroleum hydrocarbons and natural modern organic compounds, has been successfully shown to have potential for monitoring the sources and fates of organic contaminants in the marine environment (Morrill et al., 2014b; Reddy et al., 2002). Natural organic matter (NOM) contains mostly modern carbon with a $\Delta^{14}C = \sim 100 \pm 50‰$ (Petsch et al., 2001). This is because ^{14}C, a radioactive isotope of carbon, is constantly being created through the interaction of cosmic rays with atmospheric nitrogen. This ^{14}C combines with oxygen to produce carbon dioxide, which can be incorporated into plants through photosynthesis. When animals consume these plants the ^{14}C is transferred into animal biomass. When the plant, or animal, dies it stops interacting with its environment and the concentration of ^{14}C begins to decrease in its biomass (Taylor and Bar-Yosef, 2014). Petroleum hydrocarbons are millions of years old and thus contain no detectable ^{14}C due to loss by radioactive decay. Therefore, these compounds will have a $\Delta^{14}C = -1000‰$. If organisms are consuming petroleum hydrocarbons they will have a more negative $\Delta^{14}C$ signature (Morrill et al., 2014b). By comparing the $\Delta^{14}C$ of scallops or mussels from the two bays

we should be able to determine if organisms in Port au Port Bay have been exposed to more petroleum hydrocarbons then those present in St. George's Bay. These two techniques (i.e. chemical fingerprinting and radiogenic carbon measurements) combined may help differentiate between modern and ancient carbon and allow them to be used as an indicator oil contamination in organisms.

Chemical fingerprinting and molecular-level ^{14}C have both been used in other studies. Smith et al. (2009), for example, used chemical fingerprinting techniques to study the heavily industrialized Sydney Harbour, Nova Scotia (Smith et al., 2009)(Smith et al., 2009)(Smith et al., 2009)(Smith et al., 2009)(Smith et al., 2009)(Smith et al., 2009)(Smith et al., 2009)(Smith et al., 2009)(Smith et al., 2009). The harbour was an urban marine inlet that was subjected to large atmospheric and effluent inputs including metals and PAHs from a coking and steel manufacturing facility. Sediments were sampled from 41 areas around the harbour and extracted using a Soxhlet extraction method. Samples were cleaned up using a silica gel column and analyzed with a gas chromatograph equipped with a mass spectrometer detector (GC-MSD). The investigators also used ^{210}Pb dating to associate elevated PAH levels with time periods. All PAH concentration values were above 1000 μg/g (Smith et al., 2009). The minimum PAH concentration occurred in the early 1900 samples and the maximum values occurred in the samples from the 1980s. They were also able to map a spatial distribution of PAH concentrations across the harbour and project future PAH concentrations (Smith et al., 2009).

Morrill et al. (2014) applied this chemical fingerprinting technique to sediments from Hamilton Harbour, Ontario. The harbour is a freshwater environment that has been

heavily impacted by industrial activity. This project compared a heavily contaminated site near a facility used to store coal and a less contaminated site on the recreational side of Hamilton Harbour to determine if contamination from the heavily contaminated site was reaching the less contaminated site. Using chemical fingerprinting coupled with stable and radiogenic carbon isotopes there was no evidence that contamination from the heavily contaminated site was reaching the less contaminated site (Morrill et al., 2014).

A study on the Deepwater Horizon marine oil spill also employed similar chemical fingerprinting techniques (Mahmoudi et al., 2013). Sediment samples were collected from two impacted sites (evident oil residues) and two reference sites (no evident oil residues). Between 0.05-2.5 g of wet sediment were extracted by accelerated solvent extraction (ASE). The analytes in the extracts were separated based on their polarities using silica gel columns and analyzed with GC-MSD. PAH concentrations in impacted sites ranged from 16.2-99.4 mg/kg and alkane concentrations ranged from 1303-6987 mg/kg. Additionally, this study successfully used stable carbon and radiocarbon isotopes to detect metabolized fossil carbon in bacterial cell wall molecules thus demonstrating that *in situ* biodegradation of the spilled oil was occurring (Mahmoudi et al., 2013).

1.5 Experimental Approach

The goal of this thesis was to determine whether the Port au Port Bay fishing grounds were contaminated with fossil fuels and/or metals and to determine if muscles (a surrogate for scallops) were being negatively impacted. Mussels were used as proxy organisms for scallops. Mussels and scallops are both filter feeding bivalves and good

indicators of water quality in a marine environment. We had five objectives to achieve this goal:

1. Develop and test a method to successfully extract organic contaminants from sediments contaminated with crude oil;
2. Chemically characterize potential fossil fuel contaminants including diesel, gasoline, and crude oil leaking from exploration well for their organic and inorganic composition;
3. Determine if water and sediments at the fishing grounds contained organic and or inorganic contaminants found in crude oil;
4. Determine if mussels were consuming fossil fuels through ^{14}C analysis of their tissue and or metals; and
5. Determine the health of the mussels.

Chapter 2 Sampling and Analytical Methods

2.1 Sampling Locations and Dates

Two field trips were completed on the west coast of Newfoundland to Port au Port Bay and nearby St. George's Bay. The goal was to compare the geochemistry of Port au Port Bay where the fishermen report a lack of scallops and St. George's Bay where no problem has been reported. Table 2.1 lists all of the sites visited, their locations, and descriptions.

Table 2.1 Sample site names, locations, and descriptions.

Site Acronym	Site Name	Site Description	Site location	Approximate Water Depth
PBFG	Port au Port Bay Fishing Grounds	Western side of bay where scallops were once abundant	N48°36’84.0” W058°57’38.0”	30 m
PBSP	Port au Port Bay Shoal Point	Close to the point source where oil is leaking into the bay	N48°36’82.2” W058°50’57.0”	1 m
PBFW	Port au Port Bay Fishing Wharf	Along the shore of Port au Port Bay near an old fishing wharf	N48°34’79.6” W058°54’31.3”	1 m
PBSB	Port au Port Bay Sand Bar	Along the Port au Port Bay side of sand bar separating the bays	N48°33’54.3” W058°43’87.8”	1 m
GB	St. George’s Bay	Along St. George’s Bay	N48°30’48.0” W058°27’13.0”	1 m
GBSB	St. George’s Bay Sand Bar	Along the St. George’s Bay side of the sand bar	N48°27’89.1” W058°25’88.3”	1 m
GBSC	St. George’s Bay Stephenville Crossing	Along St. George’s Bay near Stephenville Crossing	N48°30’58.4” W058°26’93.3”	1 m

The first trip was completed in October 2014 at the end of the scallop fishing season. Three sampling sites were selected for this field trip: Port au Port Bay Fishing Grounds (PBFG), Port au Port Bay Shoal Point (PBSP), and St. George’s Bay (GB). The

PBFG site represents a site identified by members of the Port au Port Bay Fisheries Committee as a site where scallop beds were once abundant. Figure 2.1 identifies these sites on a local map of the area. Sediment and water samples were taken from each site for analyses. During this trip a diver also attempted to collect live scallops to be sampled. Unfortunately, no live scallops were found. On this trip no scallops could be sampled from St. George's Bay due to lack of logistical support. Based on the limited samples collected on this first trip, the sampling plan was adjusted for a second sampling trip.

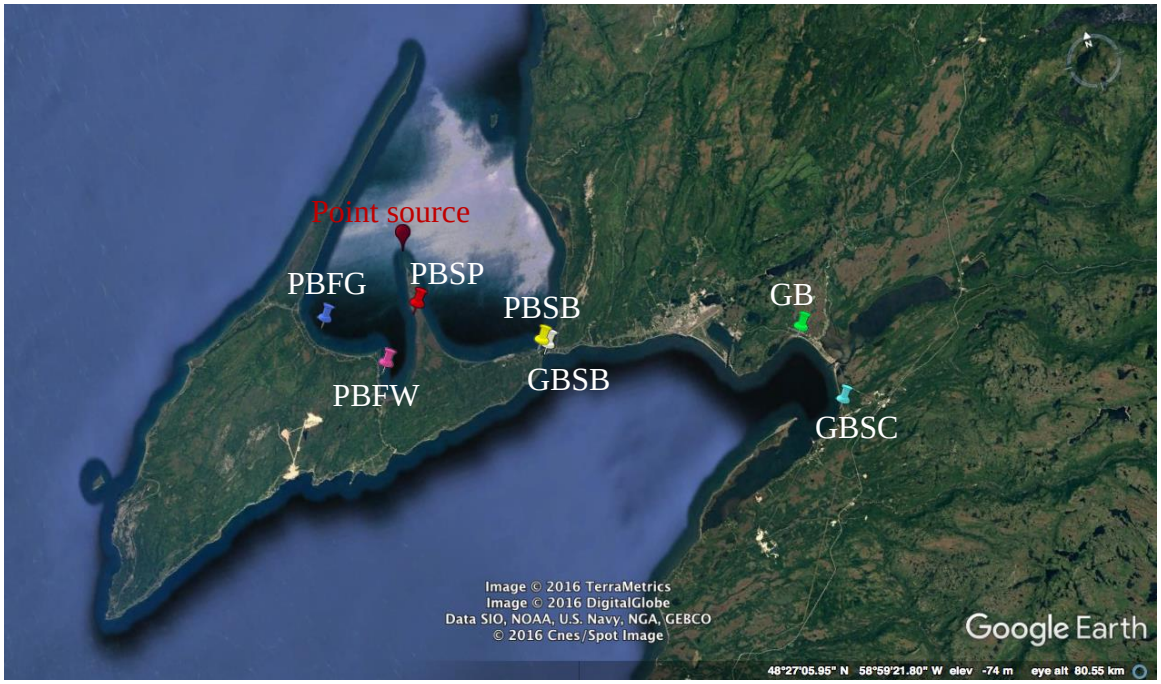

Figure 2.1 Map of sampling sites in Port au Port Bay and St. George's Bay. The oil well is indicated on the map by the "point source" marker (Google Earth 7.1.5.1557. (February 20, 2017). Port au Port Bay, NL Canada. 48°27'05.95"N, 58°59'21.80"W, Eye alt 80.55 km. SIO, NOAA, U.S. Navy, NGA, GEBCO. TerraMetrics 2016. <http://www.google.ca/earth/index.html> (Accessed February 20, 2017)).

A second field trip was planned for the start of the next scallop fishing season. The focus of this second field trip was to collect scallops; however, if no scallops were found, then other more resilient benthic filter feeders (e.g. mussels) would be collected, which would serve as scallop surrogates. The second trip was completed in July 2015. During this trip the three sampling sites from the previous trip were sampled again; as well as four additional sites (Table 2.1 and Figure 2.1) for more additional coverage of the bays' geochemistry and to potentially increase the number of organisms collected: Port au Port Bay Fishing Wharf (PBFW), Port au Port Bay Sand Bar (PBSB), St. George's Bay Sand Bar (GBSB), and St. George's Bay Stephenville Crossing (GBSC). Similarly, to the first field trip sediment and water samples were taken from each site. Only one scallop could be found on this second trip; therefore, mussels were sampled instead. Table 2.2 lists the sample types taken on each field trip and the sampling locations.

Table 2.2 Measurements and sample types taken at each sampling location during each field trip.

Sample Type	**October 2014**			**July 2015**						
	PBFG	PBSP	GB	PBFG	PBFW	PBSB	PBSP	GB	GBSB	GBSC
Temp/pH				X	X		X			
DO*				X	X	X		X	X	
Ions	X	X	X							
DOC*	X	X	X	X	X	X	X	X	X	
TIC*	X	X	X							
Sediment cores	X	X	X	X	X	X	X	X		X
Mussels					X				X	

*DO signifies dissolved oxygen, DOC signifies dissolved organic carbon, TIC signifies total inorganic carbon

2.2 Sampling Techniques

Water close to sediment was collected using a Masterflex® E/S™ portable peristaltic pump with a 50 m long Tygon® tube. At each site temperature, pH, and dissolved oxygen were measured *in situ*. Temperature and pH were measured using an Oakton handheld waterproof field probe. The probe was calibrated before each use with pH buffer solutions (4, 7, and 10) purchased from British Drug Houses (BDH). Dissolved oxygen was measured onsite using a commercially available titrating method (LaMotte Winkler Kit) following the LaMotte (2014) method. In short, water was collected in a bottle and capped underwater to ensure there is no contact with the atmosphere. Manganous sulfate and potassium iodide azide were added and a precipitate of

manganous hydroxide was formed. Sulfuric acid was then added to dissolve the precipitate and fix the sample. The solution was then titrated with sodium thiosulfate using a starch indicator solution.

For major and trace ion analysis 10 mL of sample was filtered through a 0.45 μm mixed cellulose esters (MCE) membrane filter with a sterile 60 mL syringe and collected in acid-washed plastic 15 mL falcon tubes. Samples were then preserved using 8 N nitric acid and frozen. Samples were thawed in a refrigerator just before analysis.

For dissolved organic carbon (DOC) analysis 40 mL water samples were filtered through a pre-combusted 0.7 μm glass microfiber filter (GF/F) to remove particulate matter. Samples were stored in acid-washed and pre-combusted 30 mL amber Volatile Organic Analyte (VOA) vials with Teflon-lined silica septa. Sample vials were pre-spiked with 20% phosphoric acid for preservation and stored cold and dark until analysis. DOC samples were analyzed for concentration and stable carbon isotope values ($\delta^{13}C$) at the G.G. Hatch Stable Isotope Laboratory at the University of Ottawa.

For total inorganic carbon (TIC) analysis 40 mL water samples were stored in acid-washed pre-combusted 40 mL amber VOA vials with black butyl septa and no headspace. A saturated mercuric chloride solution was used to preserve samples. Samples were stored cold and dark until analysis. TIC samples were analyzed for concentration and $\delta^{13}C$ at the G.G. Hatch Stable Isotope Laboratory at the University of Ottawa.

Sediment samples were collected from all sites in Port au Port Bay and St. George's Bay using a hand corer. The hand corer was an acid-washed, solvent rinsed 30 cm diameter Polyvinyl Chloride (PVC) tube. The sediment cores were separated into 3 sections of between 2-3 cm each using an acid-washed, solvent rinsed metal spatula. Two

cores were collected at each site. The sections were placed in acid-washed, pre-combusted 500 mL amber glass jars with Teflon-lined lids and stored in a cooler with ice. In the laboratory, samples were freeze-dried and sample jars were stored with desiccant in the dark until they were extracted for metals and organic compounds.

Mussels were collected from sampling sites in Port au Port Bay and St. George's Bay, where possible (Table 2.2). Once collected, mussels were stored in pre-combusted amber glass jars with Teflon-lined lids in a cooler with ice. Once transported to the laboratory organisms were frozen.

Crude oil from the point source was sampled by members of the Port au Port Bay Fishery Committee and sent to Memorial University, St. John's campus. Additionally, a refined petroleum sample and a diesel sample were obtained from a gas station in St. John's, Newfoundland. Samples were stored cold and dark until analysis.

2.3 Organic Extraction Method Development

Three methods of organic extraction (i.e., Soxhlet, accelerated solvent extraction (ASE), and ASE with integrated silica gel columns) were tested for their extraction efficiency for polycyclic aromatic hydrocarbons (PAHs). The Soxhlet extraction method was based on the EPA method 3540c and ASE extraction methods were adapted from Dionex Application Note 313. Reference soil (EC-1), obtained from Environment Canada, contained known concentrations of 16 common polycyclic aromatic hydrocarbons (PAHs): naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, phenanthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene,

benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,j]perylene, and indeno[1,2,3-cd]pyrene. This reference soil was used to test the three extraction methods.

For Soxhlet extraction, 2.0 g of EC-1 sediment was weighed and transferred to a new, pre-extracted cellulose thimble. The cellulose thimble was pre-extracted using a 1:1 mixture of hexane:acetone for 10 min and allowed to dry. Recovery standards (m-terphenyl, 9,10-dihydrophenanthrene, and 5-α-cholestane) were added on top of the soil sample. The thimble was placed in an acid-washed, pre-combusted Soxhlet extractor. The extractor was attached to an acid-washed, pre-combusted condenser and round bottom flask containing 300 mL of a 1:1 hexane:acetone mixture with four clean boiling chips. The sample was extracted for approximately 20 hours at a rate of 4-6 cycles/hour over medium-low heat.

A Thermo Scientific™ Dionex™ ASE™ 350 was used to extract sediment samples. ASE cells were rinsed with nanopure water and sonicated for 10 minutes. Cells and cellulose filters were then rinsed with acetone and allowed to dry. Approximately 2 g of homogenized, freeze dried sample was added to the cell and the exact weight of the sediment added was recorded. The remaining volume of the cell was filled with clean diatomaceous earth (DE). Recovery standards were added on top of the DE before cells were sealed and transferred to the ASE.

While extracts from the Soxhlet and the ASE were further cleaned up by external silica gel columns, a third extraction method was tested which combined extraction and silica gel column analyte separation in the ASE. A similar ASE method (as described above) was used; however, this time 1.5 g of activated silica gel was added to the ASE cell before the sediment, DE, and recovery standards were added. The system pressure on

the ASE was 1500 psi, and the oven temperature was set to 100°C. The oven heat up time was 5 minutes and the static time was also 5 minutes. The flush volume was 60% of the extraction cell volume and the nitrogen purge pressure was 1 MPa. An extra blank was always run prior to the sample extraction to ensure there was no carry-over from the previous analysis.

As mentioned previously, the Soxhlet and the initial ASE extracts were cleaned up using external silica gel columns before concentration. Extracts were evaporated to approximately 1 mL under a nitrogen stream with heat below 40°C. Chromotagraphy columns (40 cm long) were acid-washed and pre-combusted before packing with 4.0 grams of fully activated (400°C for 8 hours) 100-200 mesh silica gel on top of pre-combusted glass wool. Columns were eluted with 40 mL of hexane. Sample was loaded on the column and eluted with 20 mL of hexane into an acid-washed, pre-combusted Kimax tube (F1). Following the hexane fraction, the column was eluted with 21 mL of 1:2 hexane:dichloromethane (DCM) (F2), 20 mL of DCM (F3), and 20 mL of methanol (F4). All fractions were evaporated under a stream of nitrogen with heat below 40°C to a final volume of 1 mL. O-terphenyl and 5α-androstane were added to each sample vial before analysis so that analytical response factors could be calculated. Analytes of interest were determined to be below detection limit in fractions F3 and F4. Therefore, these fractions were not analyzed in subsequent extractions.

2.4 Sample Preparation

2.4.1 Organic Extractions

Port au Port Bay and St. George's Bay sediment samples were extracted for PAH and alkanes following the ASE with integrated silica gel method described above due to better reproducibility and extraction efficiency of that method (see section 3.1 in Results). Petroleum end members (crude oil, gasoline, and diesel) were extracted for their total lipids. Crude oil was also extracted for its water-available fraction (WAF).

For Total Lipid Extract (TLE) of crude oil, diesel, and gasoline, 1 g of fossil fuel was weighed out into an acid-washed, pre-combusted glass vial containing 100 mL of DCM and recovery standards (m-terphenyl, 9,10-dihydrophenanthrene, and 5-α-cholestane). This mixture was left overnight. The next day the DCM fraction was removed and reduced to approximately 1 mL under a nitrogen stream with heat below 40°C. The analytes in the extracts were separated based on their polarities using external silica gel columns as described above. The resultant fractions (F1, F2, F3, and F4) were evaporated under a stream of nitrogen with heat below 40°C to a final volume of 1 mL. O-terphenyl and 5α-androstane were added to each sample vial so that analytical response factors could be calculated.

To make the WAF of the crude oil, 3 grams of oil was weighed out into an acid-washed, pre-combusted glass vial with 27 mL of artificial seawater and allowed to mix for 18 hours following the method of Singer *et al.* (2000). Artificial seawater was prepared following the method outlined by Kester *et al.* (1967). The following day the residual non-aqueous phase liquid was removed. The remaining seawater contained the WAF of the oil. To extract the total petroleum hydrocarbons from the WAF 30 mL of DCM was added to the aqueous solution in an acid-washed, pre-combusted separatory

funnel. Recovery standards (m-terphenyl, 9,10-dihydrophenanthrene, and 5-α-cholestane) were added and the organic fraction was collected in an acid-washed, pre-combusted Kimex tube. Analytes in the extracts were separated and prepared for analysis using methods described above.

After partially thawing the mussels their tissue was removed from the shell and stored in acid-washed Teflon bottles. Mussel tissue was freeze-dried and stored in the dark. The bulk tissue was then powdered and homogenized in acid-washed, pre-combusted glass vials. The same bulk tissue was then dried in an oven at 60°C overnight before being shipped to Woods Hole Oceanographic Institution National Ocean Sciences Accelerator Mass Spectrometer (NOSAMS) laboratory for bulk organic ^{14}C analysis.

2.4.2 Sample Digestion for Metal Analysis

Sediment samples for trace metal analysis were crushed and stored in acid-washed, pre-combusted amber vials after freeze-drying. One hundred mg of sample was weighed into an acid-washed Savillex® PFA vessel. Hydrogen peroxide was added to samples to remove organic material present. Samples were then digested with successive steps of concentrated sub-boiled nitric acid and hydrofluoric acid in vessels on a hot plate at approximately 130°C for more than 48 hours. This step was followed by re-digestion with hydrochloric acid for approximately 24 hours, during which time solutions were ultra-sonicated to ensure complete digestion. Samples were dried down and diluted 500 times before analysis.

To determine the trace metal content of the crude oil that was soluble in seawater, 170.50 mg of sample was weighed out into an acid-washed plastic Falcon tube with 50

mL of artificial seawater. The next day, 10 mL of the water sample was filtered through a 0.22 μm MCE filter into acid-washed 15 mL Falcon tubes. This extraction procedure was done in triplicate. The extractions were subsequently frozen. Just before analysis, samples were thawed and acidified with 300 μL of concentrated nitric acid. Fifteen ml of the starting artificial seawater was also analyzed to determine the background concentrations of trace metals in the sample.

A method adapted from Shiel was used to digest mussel tissue samples (Shiel et al., 2012). One hundred mg of freeze-dried mussel tissue was transferred to an acid-washed Savillex® PFA vessel. Samples were then digested with successive steps of sub-boiled nitric acid, hydrofluoric acid and hydrogen peroxide in vessels on a hot plate at approximately 100°C for more than 72 hours. Samples were dried down and diluted 200 times before analysis.

2.5 Health Indices of Mussels

Mussel Health indices were determined following methods adapted from (Mohammad et al., 2015). The wet weight of frozen mussels in shell were determined. The shell height, length, and width of each mussel was determined and recorded. Mussel health indices were compared between mussels from Port au Port Bay and St. George's Bay.

2.6 Analytical Methods

DOC and TIC samples were analyzed for concentration and $\delta^{13}C$ in the G.G. Hatch Stable Isotope Laboratory at the University of Ottawa. The analytical methods

were based on Aurora 1030 Wet Oxidation TOC analyzer Operator's Manual (2005) for saltwater samples. Samples were analyzed using an OI Analytical Aurora Model 1030W TOC Analyser with a model 1088 autosampler and a combustion unit. For TIC analysis phosphoric acid is used to release the inorganic carbon. For TOC analysis, hydrochloric acid is used to release the inorganic carbon and flush it from the system. The remaining water is injected into a combustion unit and converted into carbon dioxide. The TOC analyser was interfaced to a Finnigan Mat DeltaPlusXP isotope ratio mass spectrometer for analysis by continuous flow. Internal standards were used to normalize results. The analytical precision for concentration analysis was 2% (2σ) and 0.2‰ for the $\delta^{13}C$ analysis.

Extracted sediment samples were analyzed for PAH and alkane concentrations using a gas chromatograph equipped with a mass selective detector (GC-MSD). An Agilent 6890 GC coupled to a 5973 quadrupole mass spectrometer on full scan mode was used for identification and quantification of PAHs and alkanes. The GC was equipped with a HP5-MS (30 m x 0.25 mm i.d. x 0.25 μm stationary phase thickness) column. Compounds were separated using the following temperature program: 70°C for 0.5 min, ramped to 300°C at 7°C/min and held at 300°C for 20 min. The following 16 EPA priority PAH were quantified: naphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene/triphenylene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene. O-terphenyl and 5α-androstane were added to the samples as internal standards. Recoveries were determined using external PAH and alkane standards from Sigma-Aldrich and m-terphenyl, 9,10-

dihydrophenanthrene, and 5-α-cholestane as recovery standards. PAHs and alkanes were not detected in method blanks. The detection limits for PAH and alkane analysis were below 10 μg/kg. Reproducibility on duplicate extractions was better than 6% RSD for all PAH and alkanes present in EC-1, the Environment Canada sediment standard. Analytical error (i.e. instrument precision) was less than 10% RSD.

Concentrations of trace metals in water were measured on the Perkin Elmer ELAN DRCII quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Prior to analysis, water samples and reference materials were diluted ten times with sub-boiled 0.2 M nitric acid to ensure total dissolved solids were less than 0.1% by mass. Internal standards of Sc, Rh, Re, and Th were added to monitor for instrumental drift. Data was normalized using internal standards. USGS water reference materials, sample blanks, and replicates were used for quality assurance. Blank values were negligible compared to sample values. Detection limits for ICP-MS are listed in Table 2.3. Analytical error was below ± 5% RSD.

Table 2.3 Detection limits of elements analyzed on a Perkin Elmer ELAN DRCII quadrupole Inductively Coupled Plasma Mass Spectrometer.

Element	**DL(µg/L)**	**Element**	**DL (µg/L)**	**Element**	**DL(µg/L)**
Li	0.149	Cr	0.568	Mo	0.059
Be	0.512	Fe	6.293	Ag	0.0514
B	1.520	Mn	0.034	Cd	0.097
Mg	54.545	Co	0.045	Sn	0.046
Al	0.750	Ni	0.277	Sb	0.053
Si	73.104	Cu	0.695	I	0.731
P	16.276	Zn	0.797	Cs	0.0115
S	2350	As	0.928	La	0.0072
Cl	8054	Se	6.372	Ce	0.0107
Ca	110.308	Br	15.517	Hg	0.052
Ti	5.906	Rb	0.0175	Tl	0.017
V	13.069	Sr	0.3408	Bi	0.0138
U	0.0051				

DL signifies detection limit.

Concentrations of trace metals in sediments, fossil fuel extracts, and mussel samples were analyzed using Perkin Elmer Optima 5300 DV Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). ICP-OES was used to measure trace metals for these samples when the ICP-MS was not available. Calibration standards ranging from 0.01 mg/L to 1000 mg/L were analyzed to ensure results were within the range of calibration. Reference standards were used to ensure accuracy of data. Blank and duplicate samples were analyzed for quality assurance. Blank values were negligible compared to sample values. The detection limit for ICP-OES was below 0.01 mg/L. The analytical error was less than 5% RSD.

Chapter 3 Results

3.1 Organic Extraction Method Development

Three sediment organic extraction methods were tested (see section 2.3 of methods) using the Environment Canada standard EC-1, a certified sediment standard containing 16 common PAHs: 1) Soxhlet, 2) Accelerated Solvent Extraction (ASE) with external silica gel column, and 3) ASE with internal silica gel column. When comparing the % recoveries of PAHs using each of the three methods, it was determined that a greater amount of PAHs were recovered using the ASE methods compared to the Soxhlet method (Figure 3.1). Of the two ASE methods tested (i.e. one with an integrated silica gel within the ASE cell and the other with the silica gel clean up step preformed manually after the ASE method) there was little difference in the extent of PAHs recovered. However, the PAH recoveries for the ASE + internal silica gel column method had a smaller standard deviation (i.e. the method was more reproducible) for most PAHs compared with the ASE + external silica gel method (Figure 3.2). Additionally, the ASE + external silica gel column only allowed three samples to be extracted at a time comfortably and each extraction took approximately two days and required up to 1 L of solvent per sample. The ASE + internal silica gel column allowed 20 samples to be extracted overnight without any additional manual work and reduced the volume of solvent required to approximately 30 mL per sample. For these reasons, the ASE + internal silica gel column method was chosen for the sediments in this study.

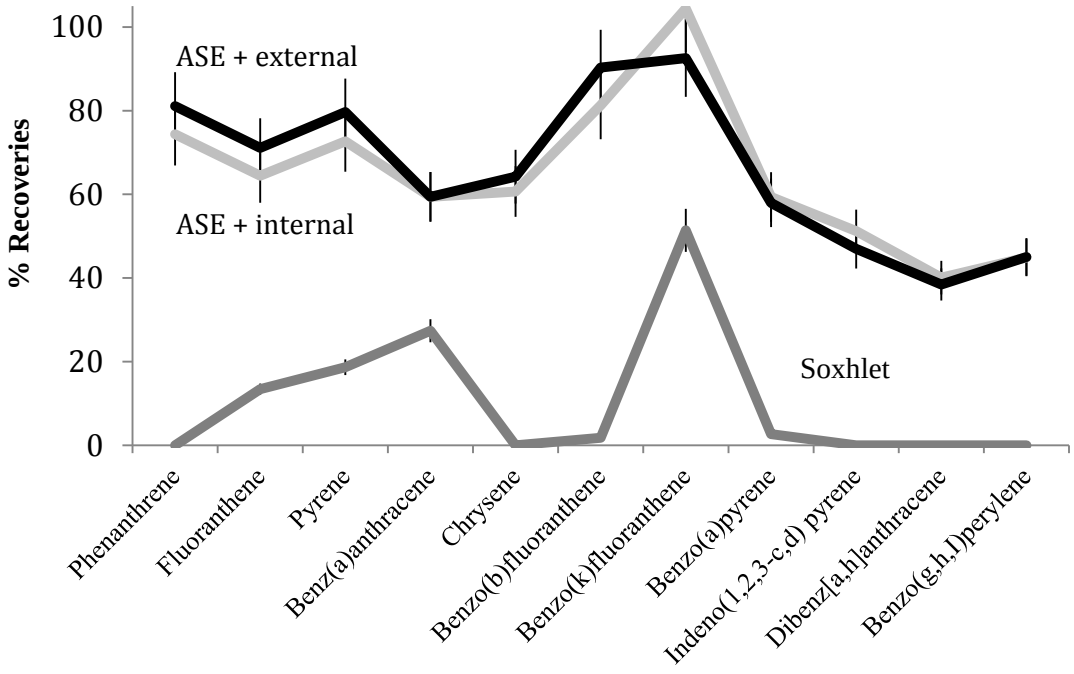

Figure 3.1 Comparison of polycyclic aromatic hydrocarbons (PAH) recoveries of Environment Canada sediment standard (EC-1) using accelerated solvent extraction (ASE) with external silica gel column, ASE with internal silica gel column, and Soxhlet extraction. The error bars represent the standard analytical error for the analysis (i.e. ±10%). Data used to generate this Figure can be found in Table A.1 of the Appendix.

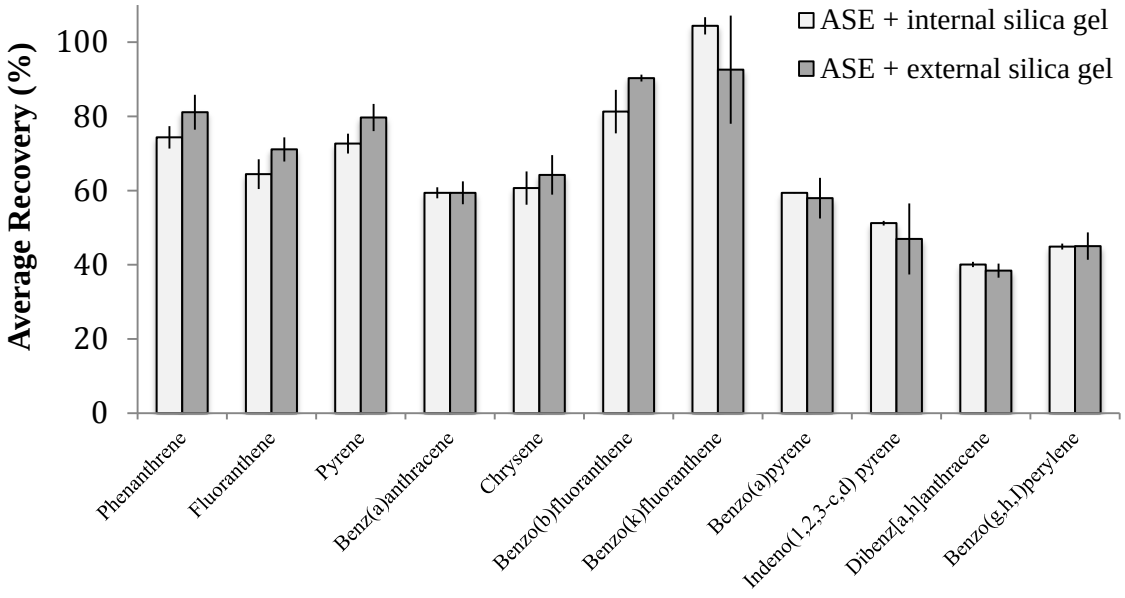

Figure 3.2 Average recoveries of polycyclic aromatic hydrocarbons (PAH) in Environment Canada sediment standard (EC-1) from Accelerated solvent extraction (ASE) with an external silica gel column and ASE with a silica gel column in the ASE cell. Error bars represent standard deviation between duplicate samples.

3.2 Aqueous Geochemistry of Port au Port Bay and St. George's Bay

Water chemistry data collected from the July 2015 trip to Port au Port Bay and St. George's Bay was summarized in Table 3.1. Port au Port Bay had higher dissolved oxygen content than did St. George's Bay. The pH of the Port au Port Bay Fishing Ground (PBFG), Port au Port Bay Fishing Wharf (PBFW) and Port au Port Bay Shoal Point (PBSP) was the same (i.e. pH = 7.1). The pH of St. George's Bay (GB) was not tested during this field trip due to sampling restrictions.

Table 3.1 Water chemistry collected during the July 2015 field trip.

	PBFG	PBFW	PBSB	PBSP	GBSB	GB
Temp (°C)	16.3	-	-	-	-	-
pH	7.1	7.1	-	7.1	-	-
DO* (mg/L)	7.2	7.4	7.4	-	6.4	6.4

Parameters not measured are signified by a dash (-).

*Dissolved oxygen is signified by DO.

The water samples collected for dissolved organic carbon (DOC) from PBSP in October 2014 had a more negative $\delta^{13}C$ value (-22.7 ± 0.3‰) than those from GB (-21.0 ± 0.2‰) and PBFG (-20.3 ± 0.2‰) (Figure 3.3 A). However, this distinction in $\delta^{13}C$ between the site sampled closest to the crude oil source site and the other sampling locations (i.e. GB and PBFG) was not observed in July 2015 (Figure 3.3 B). The DOC concentrations from the PBSP and PBFG samples (1.46 ± 0.03 mg/L) were similar to the DOC concentration sampled from St. George's Bay (1.43 ± 0.08 mg/L) (Figure 3.3) in October 2014. In July 2015 the DOC concentrations were similar to the October 2014 levels (ranging from 1.26 ± 0.03 mg/L to 1.51 ±0.05 mg/L) with the exception of GB, which had a higher concentration of 1.75 ± 0.12 mg/L.

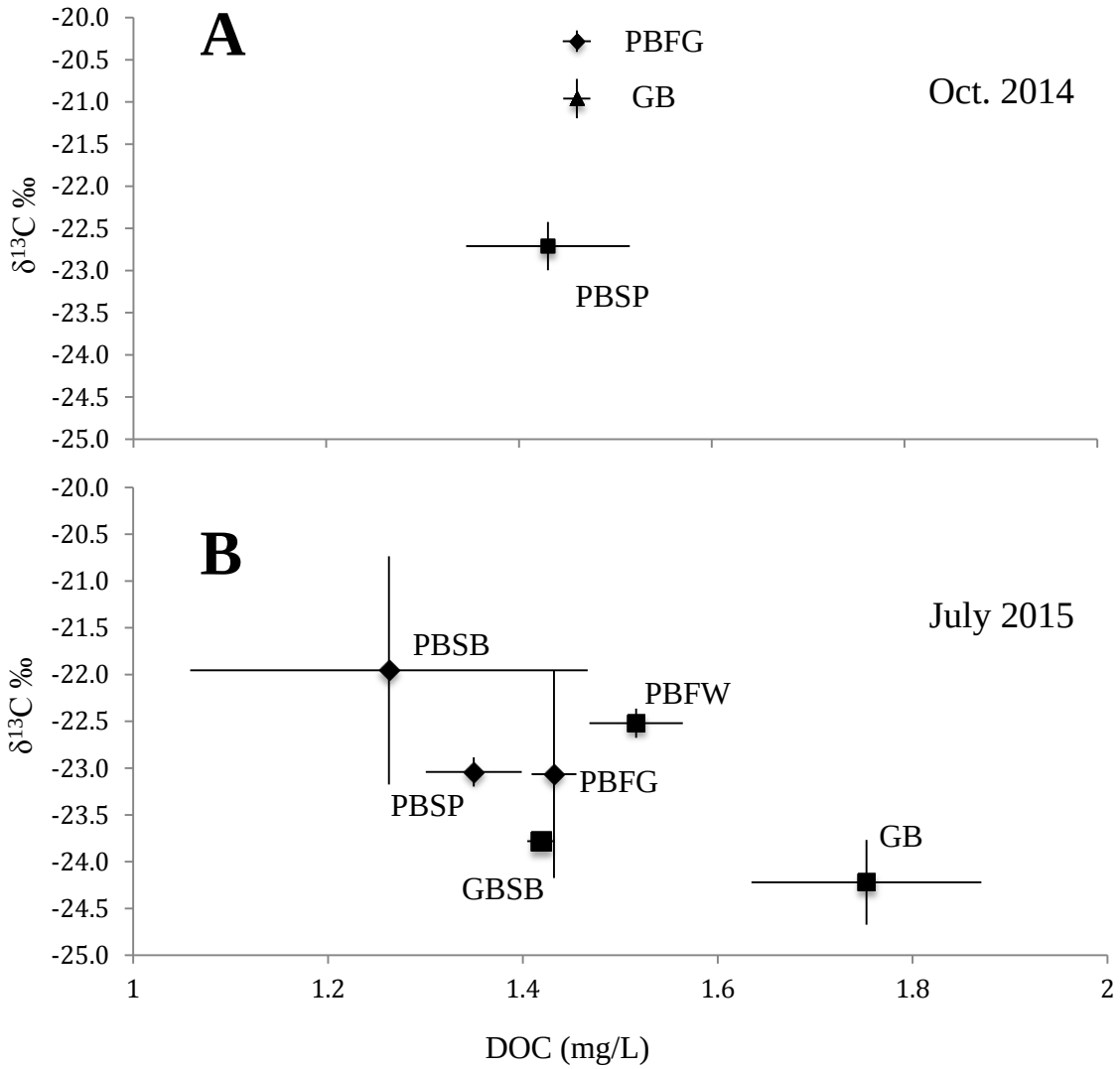

Figure 3.3 Dissolved organic carbon (DOC) data of water samples from October 2014 field trip (A) and DOC data of water samples from July 2015 field trip (B). The data points represent the average of duplicate samples. The error bars represent either the ± 1σ (standard deviation) of the average or the standard analytical error for the analysis (i.e. 2% for concentration and 0.2 ‰ for stable carbon isotope data, respectively), whichever one was greater. Data used to generate this Figure can be found in Table A.2 of the Appendix.

Total inorganic carbon (TIC) of the water samples collected from St. George's Bay in October 2014 exhibited a slightly more negative $\delta^{13}C$ value (1.1 ± 0.1‰) than those collected from Port au Port Bay Fishing Grounds (1.7 ± 0.1‰) and Port au Port Bay Shoal Point (1.5 ± 0.0‰). The TIC concentration in the samples from PBSP (27.5 ± 0.6 mg/L) was slightly higher than those collected from GB (26.1 ± 0.3 mg/L)). These results were summarized in Figure 3.4.

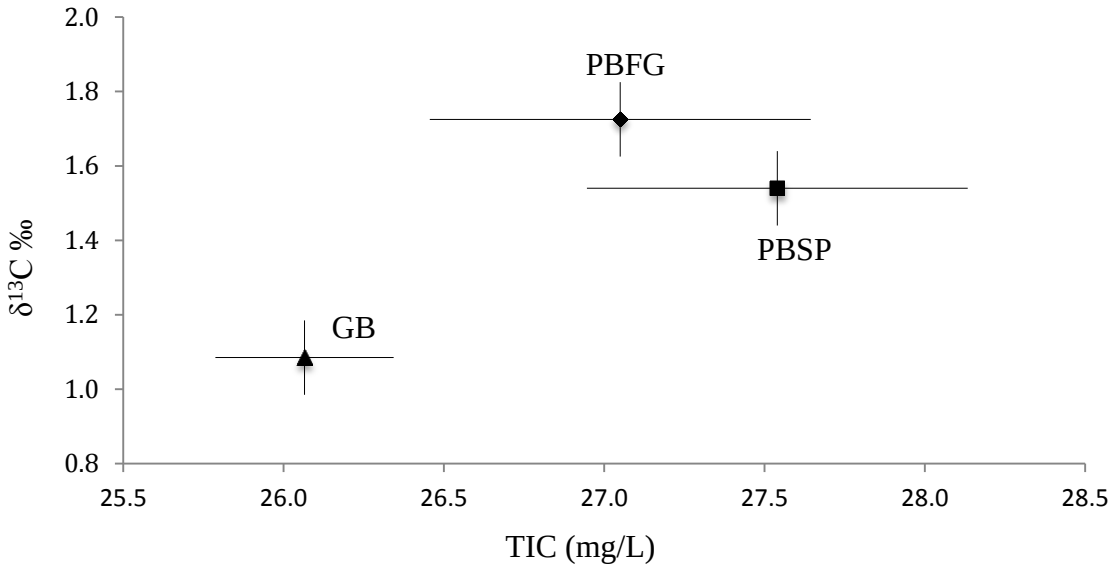

Figure 3.4 Total inorganic carbon (TIC) data of water samples from the October 2014 field trip. The data points represent the average of duplicate samples. The error bars represent either the ± 1σ (standard deviation) of the average or the standard analytical error for the analysis (i.e. 2% (2σ) for concentration and 0.2 ‰ (2σ) for stable carbon isotope data), whichever one was greater. Data used to generate this Figure can be found in Table A.3 of the Appendix.

3.3 PAH

Three potential contaminant sources: crude oil leaking from the former exploration well in Port au Port, water-associated fraction (WAF) of that crude oil, diesel, and gasoline, were extracted for their PAH content (Table 3.2). A total of four PAHs were detected in the diesel sample. Acenaphthene was the PAH present in the highest concentration in the diesel sample followed by phenanthrene and fluorene. In the crude oil and gasoline samples all 16 PAHs were below the detection limits of our analytical method (i.e. 10 μg/kg). Internal standards o-terphenyl and 5-α-androstane that were spiked into the samples before extraction had recoveries of 54.5 ± 10.3% and 53.7 ± 11.6%, respectively.

Table 3.2 Polycyclic aromatic hydrocarbons (PAHs) in crude oil, water-associated fraction (WAF) of crude oil, diesel, and gasoline samples.

	Concentration (μg /kg)			
	Crude Oil	WAF	Diesel	Gasoline
Naphthalene	<DL	<DL	<DL	<DL
Acenaphthene	<DL	<DL	1196	<DL
Fluorene	<DL	<DL	816	<DL
Phenanthrene	<DL	<DL	602	<DL
Anthracene	<DL	<DL	<DL	<DL
Fluoranthene	<DL	<DL	320	<DL
Pyrene	<DL	<DL	<DL	<DL
Benz[*a*]anthracene	<DL	<DL	<DL	<DL
Chrysene	<DL	<DL	<DL	<DL
Benzo[*b*]fluoranthene	<DL	<DL	<DL	<DL
Benzo[*k*]fluoranthene	<DL	<DL	<DL	<DL
Benzo[*a*]pyrene	<DL	<DL	<DL	<DL
Dibenz[*a,h*]anthracene	<DL	<DL	<DL	<DL
Benzo[*ghi*]perylene	<DL	<DL	<DL	<DL
Indeno[*1,2,3-cd*]pyrene	<DL	<DL	<DL	<DL

<DL signifies analyte was below detection limits of analytical method (10 μg/kg).

Sediment samples from PBFG, PBFW, PBSP, GB, and GBSC were all extracted for their PAH and alkane contents using the ASE with internal silica gel method. All 16 PAHs in the sediment samples from Port au Port Bay and St. George's Bay were below the detection limits of our analytical method (i.e. below 10 μg/kg of sediment). Internal standards m-terphenyl, 9,10-dihydrophenanthrene, and 5-α-cholestane that were spiked into the sample just before the ASE method was initiated had recoveries of 103.6 ± 15.0%, 44.9 ±29.1%, and 86.8 ± 17.5% respectively.

3.4 Alkanes

The crude oil, WAF of crude oil, diesel, and gasoline samples were extracted for their alkane content. Alkanes were detected in the crude oil sample from the PBSP ranging from 201 to 2513 mg/kg (Figure 3.5 A to D). The highest alkane concentrations in the crude oil sample were in the dodecane to heptadecane range (tridecane had the highest concentration of 2513 mg/kg). Concentrations of longer chained alkanes (i.e. greater molecular weight than heptadecane) decreased with increasing carbon number. Alkanes detected in the WAF of crude oil sample had much smaller concentrations compared to the crude oil by approximately 3 orders of magnitude. Heptadecane exhibited the highest concentration (2 mg/kg) and no alkanes with lower molecular weight than tridecane were detected. Alkanes were detected in the diesel sample primarily in the range of undecane to pentadecane (Figure 3.5) with concentrations ranging from 491 to 11,156 mg/kg. As the chain length of the alkane increased, the concentration of the alkane decreased. Alkanes were detected in the gasoline sample ranging from 25 to 75 mg/kg. Only low molecular weight alkanes were detected (i.e. the

tridecane to pentadecane range). The highest concentration alkane in the gasoline sample was tridecane (75 mg/kg). Alkanes were not identified in sediment samples from PBSP or GB (i.e. below detection limit of analytical method); however, alkanes were identified in sediment samples from PBFG (Figure 3.5 E). The alkanes identified in the sediment samples from the PBFG site were primarily in the pentadecane to heptadecane range with peak concentrations at pentadecane (2151 mg/kg, 1276 mg/kg, and 867 mg/kg in the top, middle, and bottom respectively) and decreasing concentrations from pentadecane to nonadecane. These trends were illustrated in Figure 3.5.

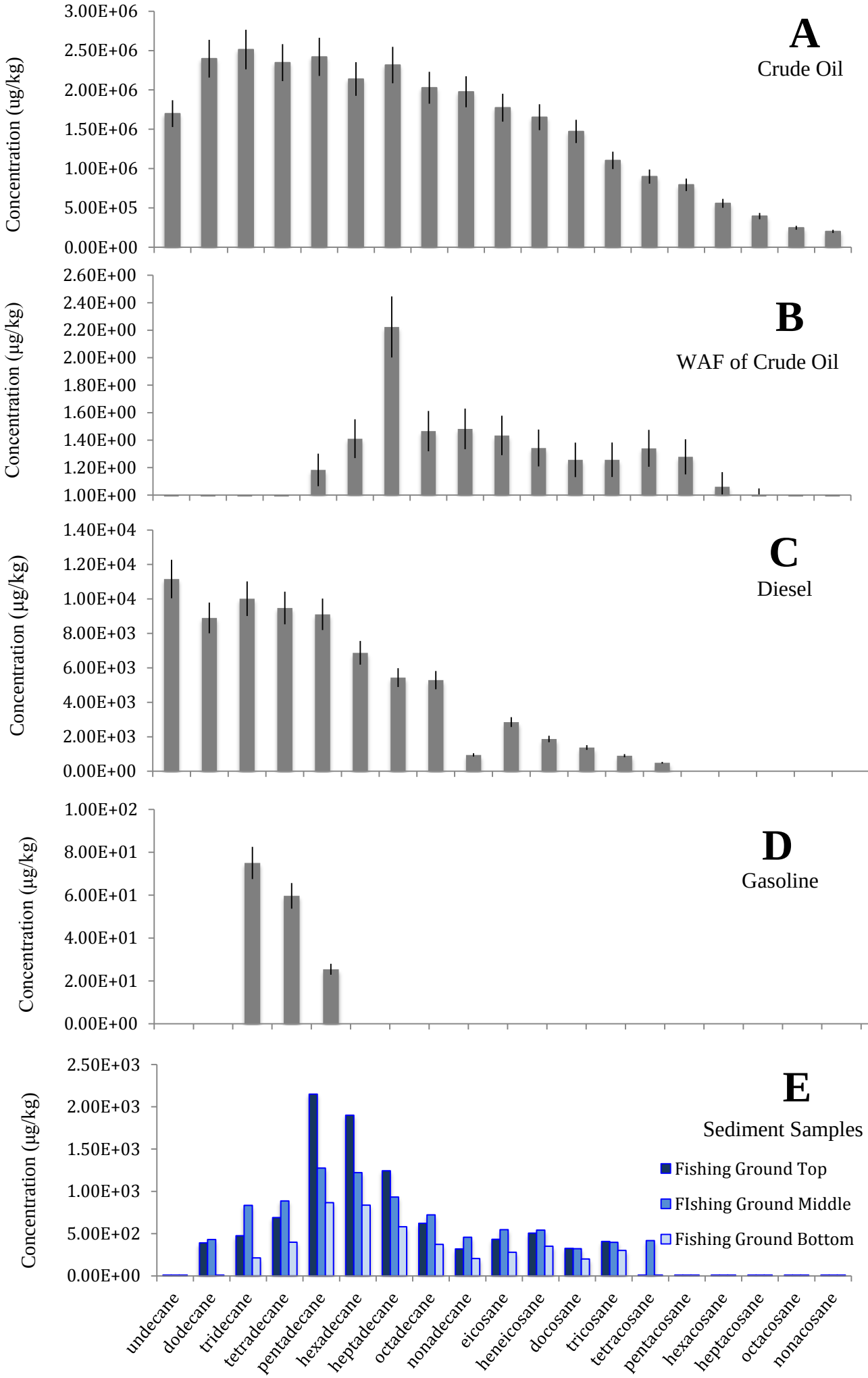
A
Crude Oil
Concentration (ug/kg)
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
B
WAF of Crude Oil
Concentration (µg/kg)
2.60E+00
2.40E+00
2.20E+00
2.00E+00
1.80E+00
1.60E+00
1.40E+00
1.20E+00
1.00E+00
C
Diesel
Concentration (µg/kg)
1.40E+04
1.20E+04
1.00E+04
8.00E+03
6.00E+03
4.00E+03
2.00E+03
0.00E+00
D
Gasoline
Concentration (µg/kg)
1.00E+02
8.00E+01
6.00E+01
4.00E+01
2.00E+01
0.00E+00
E
Sediment Samples
Concentration (µg/kg)
2.50E+03
2.00E+03
1.50E+03
1.00E+03
5.00E+02
0.00E+00
Fishing Ground Top
FIshing Ground Middle
Fishing Ground Bottom
undecane
dodecane
tridecane
tetradecane
pentadecane
hexadecane
heptadecane
octadecane
nonadecane
eicosane
heneicosane
docosane
tricosane
tetracosane
pentacosane
hexacosane
heptacosane
octacosane
nonacosane

Figure 3.5 Alkane concentrations in crude oil sample from Shoal Point (A), water-associated fraction (WAF) of crude oil from Shoal Point (B), diesel sample (C), gasoline sample (D), and sediment samples from Port au Port Bay Fishing Grounds (E). All alkanes in sediment samples from St. George's Bay and Port au Port Bay Shoal Point were below detection limits (i.e. below 10 μg/kg) of the analytical method. Data used to generate this Figure can be found in Table A.4 and A.5 of the Appendix. Error bars represent standard analytical error for the analysis (i.e. ± 10%).

3.5 Major and Trace Ions

Major and trace ion concentrations from the water collected during October 2014 field trip at PBFG, PBSP and GB were summarized in Figure 3.6 (A). The ions with the highest concentrations in all of the samples were Mg^{2+}, Ca^{2+}, Sr^{2+}, B^{3+}, and $Fe^{2+,3+}$ (in order from highest to lowest concentration). All of the water samples analyzed were below the detection limits of the analytical method for $Ni^{2+,3+}$, As^{3-}, $Tl^{+,3+}$, $Co^{2+,3+}$, and Cd^{2+}. Of all the ions measured, only $Pb^{2+,4+}$ and Zn^{2+} were higher in sample from PBFG ($4.65 \times 10^{-1} \pm 7.20 \times 10^{-2}$ mg/kg and $2.98 \times 10^{1} \pm 4.29$ mg/kg respectively) compared to GB ($1.29 \times 10^{-1} \pm 1.50 \times 10^{-2}$ mg/kg and $1.22 \times 10^{1} \pm 3.06 \times 10^{-1}$ mg/kg respectively). Hg^{2+} was below the detection limits at PBFG and GB.

Major and trace ion results for the sediment sample digestions were summarized in Figure 3.6 (B). The ions with the highest concentration were Al^{3+}, Ca^{2+}, Mg^{2+}, and $Fe^{2+,3+}$ at all three sites (i.e. PBFG, PBSP, and GB). The Li^{+} concentration was much higher at PBFG than at the other two sites (3.25×10^{2} mg/kg at PBFG, $5.62 \times 10^{1} \pm 1.46 \times 10^{1}$ mg/kg at PBSP, and $7.92 \times 10^{1} \pm 1.36 \times 10^{1}$ mg/kg at GB). As^{3-}, Sr^{2+} and $V^{3+,5+}$ were higher in GB ($2.56 \times 10^{1} \pm 5.09$ mg/kg , $2.67 \times 10^{2} \pm 2.86$ mg/kg, and $1.28 \times 10^{2} \pm$

2.42 mg/kg respectively) when compared to the other sites. The $Mn^{2+,4+}$ and $Ni^{2+,3+}$ concentration was much lower in PBFG samples (1.43×10^4 mg/kg and 1.78×10^1 mg/kg). The $Cu^{+,2+}$ and Zn^{2+} were higher in PBSP samples ($2.91 \times 10^1 \pm 2.26$ mg/kg and $1.20 \times 10^1 \pm 2.49$ mg/kg) than at the other two sites (1.78×10^1 mg/kg and 2.23×10^1 mg/kg in $Cu^{+,2+}$ and Zn^{2+} at PBFG and $1.08 \times 10^1 \pm 1.09$ mg/kg and 8.46 ± 2.72 mg/kg at GB).

The major and trace ion concentrations in crude oil sample collected from PBSP are shown in Figure 3.6 (C). The trace ions with the highest concentrations were B^{3+}, Ca^{2+}, Sr^{2+}, Mg^{2+}, and S^{2-} ($5.76 \times 10^2 \pm 1.25 \times 10^1$ mg/kg, $4.14 \times 10^4 \pm 1.10 \times 10^2$ mg/kg, $9.09 \times 10^2 \pm 4.57$ mg/kg, $1.23 \times 10^5 \pm 6.04 \times 10^2$ mg/kg, and $4.83 \times 10^5 \pm 7.839 \times 10^3$ mg/kg respectively). The Hg^{2+}, Al^{3+}, As^{3-}, Pb^{2+}, Cd^{2+} and Ag^{+} were all below the analytical detection limits (i.e. 1.00×10^{-2} mg/kg).

The highest ion concentrations in all mussel samples were $Si^{2+,4+}$, Ca^{2+}, and Mg^{2+}. Al^{3+} content was highest in mussels collected from PBFW ($1.22 \times 10^3 \pm 7.22 \times 10^2$ mg/kg compared to $1.39 \times 10^2 \pm 1.44 \times 10^2$ in mussels from GB) and Hg^{2+} and Cd^{2+} were below analytical detection limits in mussels from both sites (i.e. 1.00×10^{-2} mg/kg). The mussels from PBFW contained more $Si^{2+,4+}$ ($8.33 \times 10^3 \pm 1.68 \times 10^3$ mg/kg) than those from GB ($4.32 \times 10^4 \pm 7.54 \times 10^3$ mg/kg). These results are shown in Figure 3.6 (D)

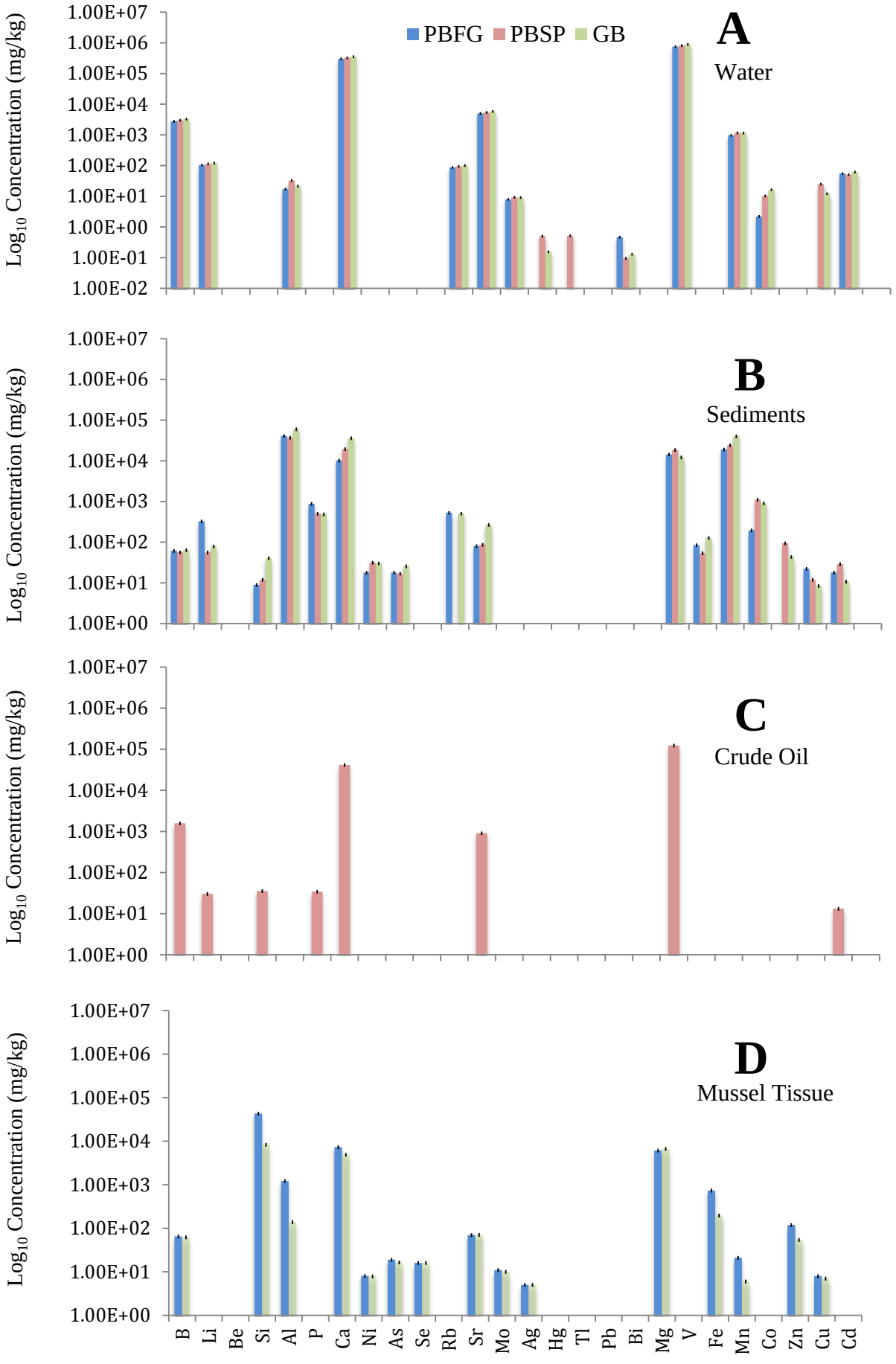
A
Water
PBFG
PBSP
GB
B
Sediments
C
Crude Oil
D
Mussel Tissue
Log10 Concentration (mg/kg)
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
1.00E-01
1.00E-02
B
Li
Be
Si
Al
P
Ca
Ni
As
Se
Rb
Sr
Mo
Ag
Hg
Tl
Pb
Bi
Mg
V
Fe
Mn
Co
Zn
Cu
Cd

Figure 3.6 Major and trace ion comparison from water samples (A), sediment samples (B), crude oil sample (C), and mussel tissue (D). Values reported are the average (± 1σ) of duplicate measurements except sediment samples from PBFG (A). Data used to generate this Figure can be found in Table A.7, A.8, and A.9, in the Appendix. Error bars represent standard analytical error for the analysis (i.e. ±5%).

3.6 Radiogenic Carbon Dating of Mussel Tissue

The $\Delta^{14}C$ of mussel tissue was analyzed to determine if the mussels were metabolizing petroleum hydrocarbons ($\Delta^{14}C$ ~ -1000‰) or NOM (~+100‰) in Port au Port and St. George's Bay (Petsch, 2001). The $\Delta^{14}C$ of the mussel tissue from Port au Port Bay (+20 ± 5‰) was indistinguishable from the $\Delta^{14}C$ of the mussel tissue from St. George's Bay (+19 ± 5‰).

3.7 Health Indices of Mussels

The wet weight, shell length, and shell width were measured for mussels collected from Port au Port Bay (PBFG) and St. George's Bay (GB). A 2-way ANOVA was then run using statistical computing environment R (version 0.00.903) on health indices. There was no significant difference found between mussel widths from Port au Port Bay and St. George's Bay (t=-0.98559, 6.5688 d.f., p=0.3592). There was also no significant difference between mussel lengths from St. George's Bay and Port au Port Bay (t=0.02166, 6.568 d.f., p=0.9834). Finally, there was no significant difference between mussel wet weights from St. George's Bay and Port au Port Bay (t=-1.2707, d.f.=6.1253, p=0.25).

The relationship between wet weight and shell length of mussels from PBFG was analyzed for GB (Figure 3.7 A). A t-test was performed on the slopes of the lines and the difference between the two sites was not found to be significantly different (p value=0.6580). The relationship of wet weight and shell width of mussels from PBFG was compared to the sample parameters measured on GB mussels (Figure 3.7 B). The difference between the two sites was not found to be significantly different (p value=0.4575). The relationship between the shell width and shell length of mussels from PBFG was compared to the same parameters on the mussels from GB (Figure 3.7 C). The difference between the two sites was not found to be significantly different (p value=0.6633).

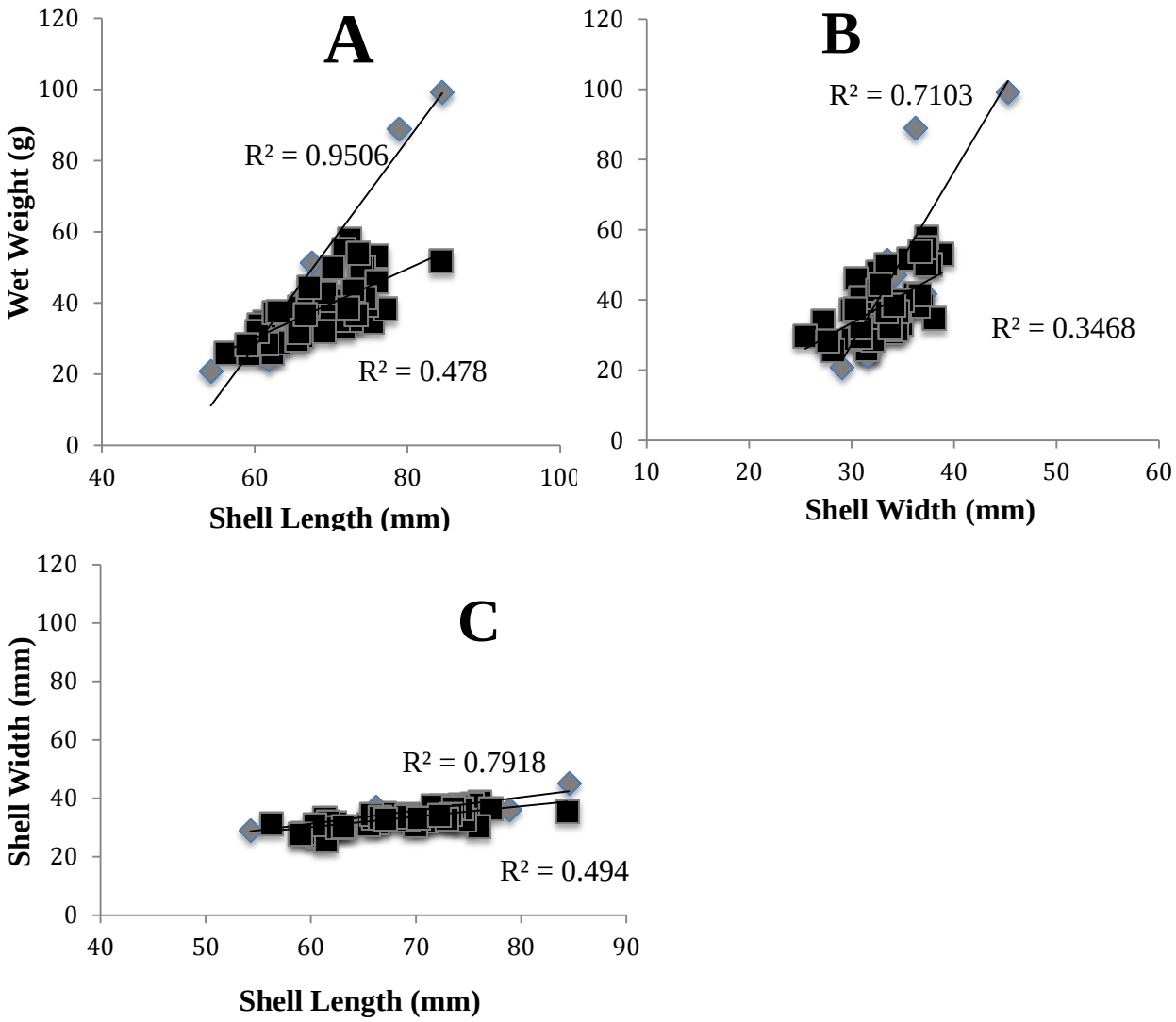

Figure 3.7 Comparison of wet weight and shell length (A), wet weight and shell width (B), and shell width and shell length (C) in mussels from Port au Port Bay (black squares) and St. George's Bay (grey diamonds). Data used to generate the Figure can be found in Table A.10 of the Appendix.

Chapter 4 Discussion

4.1 Organic Extraction Method Development

Certified reference standards (EC-1) from Environment Canada were extracted for 16 common PAHs. Three organic extraction methods were tested: 1) Soxhlet, 2) Accelerated Solvent Extraction (ASE) plus internal silica gel, and 3) ASE + external silica gel. The organic extractions completed using the ASE method had much higher recoveries (ranging from 38-82% for the 16 common PAHs) than those extracted using the Soxhlet method (ranging from 0-51% for the 16 common PAHs). In addition to increased recovery the ASE methods were also much more efficient compared to the Soxhlet method. For example, ASE extractions allowed up to 20 samples to be run over night without any labour (besides the initial sample preparation and loading) involved. Conversely, the Soxhlet method required approximately 2 days of laboratory work per sample and only 3 samples could comfortably be run at a time due to laboratory space constraints. The ASE extraction method not only saved on total extraction time, it also reduced the amount of solvent required per sample extraction. ASE extractions used less than 30 mL of solvent per sample, however, Soxhlet extractions could require up to 1 L of solvent (including the solvent required to rinse the thimble) per sample. The ASE extraction also provided flexibility with respect to the cell size in which to extract the sediment sample, and the amount of sediment extracted depending on the level of contamination.

The ASE extraction method that used internal silica gel columns reduced the amount of variation between replicates making the data more reproducible than the ASE method

with the external silica gel column. Therefore, based on increased recoveries, efficiency, and decreased solvent usage, and variability the ASE with an internal silica gel column was found to be the optimal organic extraction method and was thus used in all subsequent organic extractions.

4.2 Aqueous Geochemistry

The Port au Port Bay and St. George's Bay were very similar geographically, however, there have been no reported declines in the scallop population in St. George's Bay. Therefore, St. George's Bay was used as a background site for comparison with Port au Port Bay in this study. Geochemically, Port au Port Bay was found to be very similar to St. George's Bay. As expected, the pH values of the various locations tested within Port au Port Bay were the same (i.e. 7.1). Unfortunately, the pH of the Port au Port Bay was only tested on the later field trip and the pH of St. George's Bay and the temperature of the two bays could not be tested during both field trips due to sampling constraints. A typical pH value for a seawater environment is between 8.1-8.3 (Beer, 1996), however, the value I measured (7.1) was much more acidic. This value needs to be confirmed with more measurements and compared to the pH value of the water in St. George's Bay. Future work monitoring the temperature and pH of the two bays is necessary to determine if ocean acidification in affecting Port au Port Bay.

Dissolved oxygen (DO) content is often used an indicator of water quality. DO is low in warm waters that are high in nutrients, sediments and/or ammonia concentrations. DO is higher in colder turbulent water (Beer, 1996; CCME, 1996). Additionally, the recommended concentration of dissolved oxygen in marine and estuarine waters is 8.0

mg/L (CCME, 1996), however, the minimum required dissolved oxygen for benthic bottom-dwelling bivalves is lower (EPA, 2000). While most species of fish require a minimum of 5 mg/L of dissolved oxygen (Hitchman, 1978), some other organisms such as mussels and clams require lower concentrations but will not survive when dissolved oxygen content drops below 1-2 mg/L (EPA, 2000). Port au Port Bay and St. George's Bay had DO concentrations much higher than these minimum requirements for benthic organisms (7.2 to 7.4 mg/L and 6.4 mg/L, respectively). This data suggests that Port au Port Bay and St. George's Bay have high water quality. The DO of Port au Port Bay was slightly higher than St. George's Bay, therefore, using DO as a first approach, Port au Port Bay water quality was not lower than that of St. George's Bay.

The dissolved organic carbon (DOC) from Port au Port Bay Shoal Point (PBSP) sampled in 2014 had a more negative $\delta^{13}C$ value (-22.7 ± 0.3‰) compared to St. George's Bay (GB) (-21.0 ± 0.2‰). Oceanic DOC has an average $\delta^{13}C_{DOC}$ of -20‰ (Sharp, 2007). This more negative value observed at PBSP may have been caused by a greater contribution of crude oil to the DOC of the source site because petroleum has a $\delta^{13}C$ of approximately -25‰ (Faure, 1986; Sharp, 2007). This was the first geochemical indicator that petroleum may have been contributing to the DOC of the source site. However, this more negative value was not observed in the DOC of the Port au Port Bay Fishing Grounds (PBFG) (-20.3 ± 0.2‰). Therefore, if petroleum was contributing to the DOC at the source site, there was no isotopic evidence of it contributing to the DOC at the PBFG which had a $\delta^{13}C_{DOC}$ signature more similar to the $\delta^{13}C_{DOC}$ signature of GB. In July 2015 the $\delta^{13}C$ of the DOC at the PBSP site was no longer more negative than the DOC at the other sites. In fact, the $\delta^{13}C_{DOC}$ values of all Port au Port Bay sites were

indistinguishable. Whatever caused the more negative $\delta^{13}C$ value at the PBSP site in 2014 was no longer contributing to the DOC samples in July 2015.

If the $\delta^{13}C$ signature of petroleum hydrocarbons was not detected in the $\delta^{13}C$ signature of the DOC, this may have been because the petroleum was being oxidized to CO_2. If this were the case, then this may be observed in more negative $\delta^{13}C$ TIC data. The PBSP, and PBFG sites had slightly more positive $\delta^{13}C_{TIC}$ values (1.7 ± 0.1‰ and 1.5 ± 0.0‰ respectively). Additionally, these are within the typical range of $\delta^{13}C$ of carbon at the surface of the ocean (1 to 1.5‰: (Sharp, 2007). Therefore, there is no observable indication in the stable carbon isotope data that petroleum hydrocarbons were being oxidized to CO_2 in the PBSP or PBFG sites.

If fossil fuels are contaminating Port au Port Bay and not St. George's Bay, then a more negative $\delta^{13}C_{DOC}$ values in samples from Port au Port Bay should be observed relative to samples from St. George's Bay. The $\delta^{13}C_{DOC}$ value should also be more negative at the point source, radiating outwards into the fishing grounds. With the exception of a slightly more negative $\delta^{13}C_{DOC}$ value at the PBSP site in 2014, this trend was not observed. This may have been due to the oxidation of petroleum hydrocarbons to CO_2. However, if this process was occurring then a more negative $\delta^{13}C_{TIC}$ value may have been expected in the Port au Port Bay sites compared to the St. George's Bay site. This was also not observed. Therefore, if petroleum was contaminating the PBFG site then the concentration was not great enough to see a change in the $\delta^{13}C_{DOC}$ or $\delta^{13}C_{TIC}$ data. This begs the question how much would it take to see a change in $\delta^{13}C_{DOC}$ or $\delta^{13}C_{TIC}$? The fraction of petroleum needed to decrease the $\delta^{13}C_{DOC}$ by 1‰ can be estimated using a simple isotope mass balance (Equation 4.1):

$$\delta^{13}C_{mix} = \delta^{13}C_{petro} \times f_{petro} + \delta^{13}C_{ocean} \times (1-f_{petro}) \quad [4.1]$$

Where $\delta^{13}C_{mix}$ is the $\delta^{13}C_{DOC}$ of the bulk sample, $\delta^{13}C_{petroleum}$ is the $\delta^{13}C_{DOC}$ of the petroleum (set to -25‰), $\delta^{13}C_{ocean}$ is the $\delta^{13}C_{DOC}$ in the ocean (-20‰), and f_{petro} is the fraction of petroleum contributing to the bulk sample. Using this method, it was estimated that at least 20% of the DOC would have to be from a petroleum source for there to be a 1‰ decrease in the overall $\delta^{13}C_{DOC}$ value.

Similarly, the fraction of oxidized petroleum needed to decrease the $\delta^{13}C_{TIC}$ by 1‰ can be estimated using the same isotope mass balance (Equation 4.1). However, in the case of TIC, $\delta^{13}C_{mix}$ is the $\delta^{13}C_{TIC}$ of the bulk sample, $\delta^{13}C_{petro}$ is the $\delta^{13}C$ of the oxidized petroleum (set to -25‰, assuming no isotopic fractionation effects during oxidation), $\delta^{13}C_{ocean}$ is the $\delta^{13}C_{TIC}$ in the surface ocean (set to +1.25‰) and f_{petro} is the fraction of carbon contributing to bulk TIC from the oxidation of petroleum. Using this method, it was estimated that at least 4% of the bulk TIC would have had to come from the oxidation of petroleum for there to be a 1‰ decrease in the bulk $\delta^{13}C_{TIC}$ value. This means that at most 1.5 mg/L of TIC was derived from petroleum.

4.3 PAH

Polycyclic aromatic hydrocarbons (PAHs) are major components of crude oil that can have toxic effects on bivalves including reduced function of the immune system and impaired cellular response. Curiously, polycyclic aromatic hydrocarbons (PAHs) were not detected in the crude oil sample from the leaking oil well on Shoal Point, the water-associated fraction of the crude oil, or the gasoline sample despite having 54.5 ± 10.3% and 53.7 ±11.6% recoveries of internal standards. It is likely that no PAHs were detected

in the gasoline sample due to the refining process which separates compounds by molecular weight, resulting in a lower carbon chain range, usually around 6 carbon atoms (Fahim et al., 2010). There are several reasons PAHs could be absent from the crude oil sample. It is possible there were no PAHs in the crude oil sample initially. Pampanin & Sydnes (2013) summarized the chemical composition of 48 different crude oils and found that the concentration of PAHs present in a crude oil can vary from below the detection limit to 3,700 mg/kg. In fact, certain crude oils only contained naphthalene, fluroene, chrysene and all other PAHs were completely absent (Pampanin and Sydnes, 2013). The PAHs could also have volatilized or could have been degraded (i.e. photolysis or microbial degradation) due to exposure of the crude oil to the marine environment. A total of 4 PAHs (acenaphthene, fluorene, phenanthrene, and fluoranthene) were detected in the diesel sample. It is not surprising that we found PAHs in the diesel sample as the refining process results in a heavier carbon range (typically between 14 to 20 carbon atoms) and thus a higher density (Fahim et al., 2010). It is surprising that we only found 4 PAHs in the diesel sample, however, given that the molecular weight of these compounds is quite low relative to the other PAHs not detected, it is possible that the refining process removed these higher molecular weight compounds (Table 4.1). These 4 PAHs also have smaller K_{ow} and higher vapour pressures values relative to the other PAHs (Table 4.1) so this could also help explain why only four PAHs were detected in the diesel sample (Canada, 1994).

Table 4.1 Physical Properties of Common Polycyclic Aromatic Hydrocarbons (taken from Environment Canada, 1994).

PAH	Molecular weight (g/mol)	Log K_{ow}	Water solubility at 25°C (mg/L)	Vapour pressure at 25°C (mPa)
naphthalene	128.16	3.5	31.7	11 960
acenaphthene	154.21	4.33	3.42	3.42
fluorene	166	4.18	1.98	94.7
phenanthrene	178.24	4.5	1.29	90.7
anthracene	178.24	4.5	0.045	25
pyrene	202.26	4.9	0.135	91.3×10^{-6}
fluoranthene	202.26	5.1	0.26	1328
benz[*a*]anthracene	228	5.6	0.0057	14.7×10^{-3}
benz[*a*]pyrene	252.32	6.0	0.0038	0.37×10^{-6}
benzo[*b*]fluoranthene	252.32	6.06	0.014	0.13×10^{-5} - 0.133 (20°C)
benzo[*j*]fluoranthene	252.32	N/A	N/A	N/A
benzo[*k*]fluoranthene	252.32	6.06	0.0043	2.8×10^{-9}
indeno[*1,2,3-cd*]pyrene	276	6.4	0.00053	1.3×10^{-5}

N/A signifies information not available

Sediment was sampled from PBFG, PBFW, PBSP, GB, and GBSC. No sediment sample was taken at PBSB or GBSB sites due to logistical reasons. No PAHs were

detected in any sediment samples obtained from Port au Port Bay or St. George's Bay, however, recovery standards of m-terphenyl, 9,10-dihydrophenanthrene, and 5-α-cholestane were used (the recoveries were 103.6 ± 15.0%, 44.9 ±29.1%, and 86.8 ± 17.5% respectively). It is not surprising that no PAHs were detected in the sediment samples from Port au Port Bay since no PAHs were detected in the crude oil sample, PAHs were not the best indicator of petroleum hydrocarbon contamination in the bay.

The detection limits of our method were 10 μg/kg. Assuming the worst percent recovery of 42%, at most the sediments from Port au Port would have had less than 24 μg of PAH per kg of sediment. Therefore, all sediment samples obtained from both the St. George's Bay and Port au Port Bay were safely below the CCME guidelines for marine sediments (Table 4.2). The lowest concentration in the CCME guidelines is for acenaphthene, 88.9 μg/kg, approximately 3.7 times higher than maximum calculated PAH concentration. Additional work could be done to optimize the ASE technique (i.e. solvents used, temperature program) to consequently lower the detection limits. However, this would not change the conclusions of this study.

Table 4.2 Canadian Council of Ministers of the Environment (CCME) Guidelines for Contaminants in Marine Sediment.

PAH	µg/kg	PAH	µg/kg
Naphthalene	391	Benz(a)anthracene	693
Acenaphthylene	128	Chrysene	846
Acenaphthene	88.9	Benzo(a)pyrene	763
Fluorene	144	Indeno(1,2,3-c,d) pyrene	
Phenanthrene	544	Dibenz[a,h]anthracene	135
Antracene	245	Pyrene	1398
Fluoranthene	1494		

(Source: http://www.ccme.ca/en/resources/canadian_environmental_quality_guidelines/)

4.4 Alkanes

Alkanes were detected in the crude oil sample from the leaking oil well at Shoal Point and the water-associated fraction (WAF) of the crude oil. The alkane concentrations measured in the WAF, however, were much lower than the concentrations measured in the crude oil sample by approximately 3 orders of magnitude. This implies the alkanes are very insoluble and are likely not dissolving in the water phase in large quantities. This is not a surprising result as alkanes have very low polarity and thus limited solubility in polar solvents like water (Arora, 2006). The ocean has a greater ionic strength than freshwater and thus non-polar compounds (Hadfield and Wang, 2003), such as alkanes, are even less soluble than their K_{OW} would predict.

Alkanes were also detected in the sediment sample from the Port au Port Fishing Grounds. The most recent layer of sediments (i.e. top) contained the highest concentration of alkanes. The oldest layer of sediments (i.e. bottom) contained the lowest concentration of alkanes. This is not surprising as biodegradation (i.e. microbial degradation under aerobic and anaerobic conditions) can occur over time decreasing the alkane concentrations. Since alkanes can have natural sources it is possible that the level of alkanes from the environment simply decreased over time through microbial degradation. Alternatively, the newest sediments represent a more modern time frame (i.e. more industrial and thus more fuel usage) so it is possible that the alkanes could represent an anthropogenic source. This, however, is purely speculative and to determine if the alkanes do in fact represent an anthropogenic input, more research would be required. Due limited sediment core length Pb-210 dating was not possible; however, future work with a dating technique would prove useful in helping identify potential alkane sources. Additionally, more work in determining if microbial degradation of alkanes from the leaking abandoned oil well is occurring would be beneficial in determining the source of the alkanes.

The alkane composition of the sediment samples did not match the crude oil, WAF, or gasoline; however, the composition did bear some resemblance to that of the diesel. The diesel used in fishing boat engines could be contributing, in part, to the alkanes observed in the fishing grounds; however, the diesel alone cannot explain the alkane signature observed. The concentration of alkanes present in the diesel sample was 2 fold more than that detected in the sediment samples from PBFG. This could be explained by the solubility of alkanes; alkanes are non-polar compounds resulting in very limited

solubility in water (Arora, 2006), so it is not surprising that the concentration of alkanes in the sediment was lower than what was present in the sediment. Both the alkanes in the sediment sample and diesel sample exhibited similar trends for tridecane, tetradecane, and heptadecane (i.e. they all had similar trends in concentrations in the sample). The alkane concentrations in both samples dramatically decreased in compounds with a molecular weight greater than nonadecane. However, the sediment sample had large peaks in pentadecane and hexadecane concentrations that were not observed in the diesel sample. Perhaps these trends could be explained by microbial degradation of the alkanes, however, more research is required.

United Nations Environment Programme (UNEP) guidelines suggest that alkane concentrations in unpolluted marine sediments should not exceed 10 μg/kg (UNEP, 1995). All sediment samples exhibit alkane concentrations below 2.5 μg/kg. Generally, alkanes containing fewer than 20 carbon atoms are associated with ocean bacteria and algae. These hydrocarbons are characterized by an even carbon number dominance (Iwegbue et al., 2016). Compounds with longer carbon chains (i.e C_{10} to C_{35}) with no odd-even dominance are generally derived from fossil fuels and their combustion residues (Iwegbue et al., 2016). I only analyzed for C_{11} to C_{29}. The sediment samples had the highest concentration of C_{15}, C_{16} and C_{17} with no clear even dominance. Since my data shows that the alkanes present in highest concentrations contain fewer than 20 carbon atoms, this could suggest a biological source not a hydrocarbon source, unfortunately, however, with the standard error it is not possible to determine if there was an even dominance. Future work should be focused on determining alkane isoprenoids to assist in determining if the alkanes are biological in origin.

4.5 Major and Trace Ions

The predominant ions in the water column in the Port au Port Bay and St. George's Bay were Ca^{2+}, Mg^{2+}, S^{2-}, Sr^{2+}. All four ions are major constituents of seawater (Kester et al., 1967), so this result was not unexpected. $Pb^{2+,4+}$ was higher in samples collected from PBFG which could be explained by the higher concentration of $Pb^{2+,4+}$ in the sediment samples from the area. Hg^{2+} was below the detection limits at PBFG and GB, however, at PBSP the Hg^{2+} concentration in one sample was 0.52 mg/kg. This Hg^{2+} concentration was not reflected in the duplicate sample or in samples from the July 2015 field trip. All heavy metals were below CCME guidelines for marine water or detection limit of analytical method (Table 4.3). Therefore, no evidence of metal contamination was observed in the water column in Port au Port Bay that could explain the decline in the scallop population.

Table 4.3 Canadian Council of Ministers of the Environment (CCME) Guidelines for Contaminants in Marine Water.

Metal	µg/kg
As^{3-}	12.5
Cd^{2+}	0.12
$Cr^{2+,3+}$	ND
$Cu^{+,2+}$	ND
$Pb^{2+,4+}$	ND
Hg^{2+}	0.016
Zn^{2+}	ND

(Source: http://www.ccme.ca/en/resources/canadian_environmental_quality_guidelines/)
ND = No data available

Both St. George's Bay and Port au Port Bay have a predominance of shales and limestones (Hicks and Owens, 2014), explaining the high Ca^{2+}, Al^{3+}, and $Si^{2+,4+}$ content. $Pb^{2+,4+}$ was highest in sediments collected from PBFG which could be explained by the presence of Lead Cove in Port au Port Bay. Lead Cove is a site of lead mineralization (galena) in the East Bay portion of Port au Port Bay (Wardle, 2000). Port au Port Bay also had a higher iron content which could be explained by the presence of pyrite in Port au Port Bay (Hinchey et al., 2015). All heavy metals were below CCME guidelines for contaminants in marine sediment (Table 4.4).

Table 4.4 Canadian Council of Ministers of the Environment (CCME) Guidelines for Contaminants in Marine Sediment.

Metal	μg/kg
As^{3-}	4.16×10^4
Cd^{2+}	4.20×10^3
$Cr^{2+,3+}$	1.60×10^5
$Cu^{+,2+}$	1.08×10^5
$Pb^{2+,4+}$	1.12×10^5
Hg^{2+}	7.00×10^2
Zn^{2+}	2.71×10^5

(Source: http://www.ccme.ca/en/resources/canadian_environmental_quality_guidelines/)

The crude oil sample was mostly composed of B^{3+}, Ca^{2+}, Sr^{2+}, and Mg^{2+}. This is similar to the composition of the water sample, which is not surprising as the sample was taken from the ocean near the leaking abandoned exploration well. Trace heavy metals often found in crude oil include $Ni^{2+,3+}$, $V^{3+,5+}$, $Cu^{+,2+}$, Cd^{2+}, and $Pb^{2+,4+}$ (Osuji and Onojake, 2004); however, our crude oil sample only contained detectable amounts of copper. The amount of copper present was below CCME guidelines for contaminants in marine sediments (1.08×10^5 μg/kg). There was also no detectable Hg^{2+}, Ag^{+}, or As^{3-} in our crude oil sample.

Mussel samples contained the highest concentrations of $Si^{2+,4+}$, Ca^{2+}, and Mg^{2+}. These ions are all major components of seawater so it is not surprising that these concentrations are reflected in the mussel tissues. The mussel from the PBFW had a higher Al^{3+} content than the mussel from GB. This agrees with the ion results from the

water and sediment samples and is likely a result of the mussel feeding on sediments in the area which have a higher Al^{3+} content.

4.6 Radiogenic Carbon Dating of Mussel Tissue

The $\Delta^{14}C$ of mussels was analyzed to determine if the mussels were metabolizing petroleum hydrocarbons which are millions of years old and have no detectable ^{14}C ($\Delta^{14}C$ ~ -1000‰) or modern carbon in the form of natural organic matter (~+100‰) in Port au Port and St. George's Bay. If the mussels in Port au Port Bay were consuming more petroleum hydrocarbons they should have had a more negative $\Delta^{14}C$ signature than the mussels in St. George's Bay. The $\Delta^{14}C$ of the mussels from Port au Port Bay (+20 ± 5‰) was indistinguishable from the $\Delta^{14}C$ of the mussels from St. George's Bay (+19 ± 5‰). This suggested that the mussels in Port au Port Bay have not been consuming more ancient hydrocarbons than those of St. George's Bay. While this does not completely eliminate the possibly that mussels in Port au Port Bay were absorbing petroleum hydrocarbons, it implies that the decline in Port au Port Bay over St. George's Bay cannot be explained by the what the mussels were absorbing (i.e. the crude oil).

If mussels were consuming petroleum hydrocarbons then the concentration was not great enough to see a change in the $\Delta^{14}C$ of their biomass. To determine how much it would take to see a change in the $\Delta^{14}C$ the fraction of petroleum required to decrease the $\Delta^{14}C$ by 1‰ can be estimated using a simple isotope mass balance (Equation 4.2):

$$\Delta^{14}C_{mix} = \Delta^{14}C_{fossil} \text{ x } (f_{fossil}) + \Delta^{14}C_{mod} \text{ x } (1- f_{fossil}) \qquad [4.2]$$

Where $\Delta^{14}C_{mix}$ was the measured $\Delta^{14}C$ value of the mussel tissue, $\Delta^{14}C_{fossil}$ was set to -1000‰ and $\Delta^{14}C_{mod}$ was set to 100‰ (Petsch, 2001). The fraction of modern carbon

(f_{mod}) was estimated by substituting (1-f_{mod}) for the fraction of fossil carbon (f_{fossil}) into Equation 4.2 and solving for f_{mod}. Using this method, it was estimated that 0.1% of the $\Delta^{14}C$ would have to be from a petroleum source for there to be a 1‰ decrease in the overall $\Delta^{14}C$ value.

Using this information, we cannot say that mussels from PB are not consuming ancient carbon from a petroleum hydrocarbon source, however, we can say they are not consuming more petroleum hydrocarbons than mussels in GB.

4.7 Health Indices of Mussels

No significant difference was found in the wet weight, shell length, or shell width in mussels from Port au Port Bay compared to mussels from St. George's Bay. No significant difference was found in the wet weight *vs* shell length, wet weight *vs* shell width, and shell width *vs* shell length of mussels from Port au Port Bay compared to mussels from St. George's Bay. This suggests there was no significant difference in mussels from Port au Port Bay compared to mussels from St. George's Bay. Mussels from Port au Port Bay do not exhibit signs of poor health, suggesting mussels from Port au Port Bay are not less healthy than those from St. George's Bay.

4.8 Conclusions

All the parameters tested did not suggest that the crude oil leaking from the abandoned exploration well on the west side of Shoal Point was reaching the Port au Port Bay fishing grounds and affecting the mussel population. Additionally, the crude oil did

not appear to have any PAHs present, or if they were once present they have since been lost. Mussels are often used as indicators of water quality and have been shown to reflect the level of contamination they are exposed to in the water column (Burns and Smith, 1981). Using mussels as a proxy for scallops, since no contamination was detected in mussels sampled from the bay, suggested that decline of the scallop fishery in Port au Port Bay, Newfoundland cannot be explained by the leaking oil well at Shoal Point. Further research is required to suggest a possible explanation for the decline of the scallop population. The invasive species, the Green crab, which has been reported in literature as close to the area as St. George's Bay (DFO, 2016) and has been sighted in Port au Port Bay by locals should be investigated to determine if they could be impacting bivalves in the Bay. Ocean acidification due to climate change should also be further explored as other scallop fisheries in Canada (such as British Columbia) have suggested ocean acidification as a cause of scallop population decline (Hume, 2014). Annual monitoring of the pH and temperature of the St. George's and Port au Port bays would be useful in determining if these environmental factors could be contributing to the decline of the scallop fishery in Port au Port Bay, Newfoundland.

Appendix

Table A.1 Comparison of Polycyclic Aromatic Hydrocarbons (PAH) in Soxhlet, Accelerated Solvent Extraction (ASE) + External Silica Gel Column, and ASE + Internal Silica Gel Column

Compound	Soxhlet*	ASE + External silica gel	ASE + Internal silica gel
	Average Recovery (%)		
Phenanthrene	0	81.1 ± 4.7	74.3 ± 3.0
Fluoranthene	13.4	71.1 ± 3.2	64.4 ± 4.0
Pyrene	18.7	79.7 ± 3.7	72.7 ± 2.7
Benz(a)anthracene	27.4	59.4 ± 3.1	59.4 ± 1.5
Chrysene	0	64.2 ± 5.3	60.7 ± 4.5
Benzo(b)fluoranthene	1.8	90.3 ± 0.9	81.3 ± 5.9
Benzo(k)fluoranthene	51.4	92.6 ± 14.6	104.4 ± 2.3
Benzo(a)pyrene	2.7	58.0 ± 5.5	59.4 ± 0.1
Indeno(1,2,3-c,d) pyrene	0	47.0 ± 9.6	51.2 ± 0.6
Dibenz[a,h]anthracene	0	38.4 ± 1.9	40.1 ± 0.7
Benzo(g,h,I)perylene	0	45.0 ± 3.7	44.9 ± 0.8

* = sample does not represent an average

Table A.2 Dissolved Organic Carbon (DOC) data

	October 2014		July 2015	
	DOC (mg/L)	$\delta^{13}C_{DOC}$ (‰)	DOC (mg/L)	$\delta^{13}C_{DOC}$ (‰)
PBFG	1.46	-20.19	1.43(±0.20)	-23.07(±1.22)
PBFW	N/A	N/A	1.52(±0.05)	-22.52(±0.16)
PBSB	N/A	N/A	1.26(±0.03)	-21.96(±1.11)
PBSP	1.43	-22.71	1.35(±0.05)	-23.04(±0.16)
GBSB	N/A	N/A	1.42(±0.00)	-23.78(±0.06)
GB	1.46	-20.96	1.75(±0.12)	-24.22(±0.45)

Table A.3 Total Inorganic Carbon (TIC) data

	TIC (mg/L)	$\delta^{13}C_{TIC}$ (‰)
PBFG	1.73(±0.02)	27.1(±0.6)
PBSP	1.54(±0.00)	27.5(±0.6)
GB	1.09(±0.08)	26.1(±0.3

Table A.4 Alkane concentrations in Crude oil, water-associated (WAF) of crude oil, Diesel, and Gasoline

	Concentration (μg/kg)			
	Crude Oil	WAF of Crude Oil	Diesel	Gasoline
Undecane	1.70×10^6	< DL	1.12×10^7	1.70×10^6
dodecane	2.40×10^6	< DL	8.90×10^6	2.40×10^6
tridecane	2.51×10^6	< DL	1.00×10^7	2.51×10^6
tetradecane	2.35×10^6	3.11×10^2	9.47×10^6	2.35×10^6
pentadecane	2.42×10^6	1.18×10^3	9.11×10^6	2.42×10^6
hexadecane	2.14×10^6	1.41×10^3	6.87×10^6	2.14×10^6
heptadecane	2.32×10^6	2.22×10^3	5.44×10^6	2.32×10^6
octadecane	2.03×10^6	1.47×10^3	5.29×10^6	2.03×10^6
nonadecane	1.98×10^6	1.48×10^3	9.46×10^5	1.98×10^6
eicosane	1.77×10^6	1.43×10^3	2.85×10^6	1.77×10^6
heneicosane	1.65×10^6	1.34×10^3	1.87×10^6	1.65×10^6
docosane	1.47×10^6	1.26×10^3	1.37×10^6	1.47×10^6
tricosane	1.10×10^6	1.26×10^3	9.02×10^5	1.10×10^6
tetracosane	8.98×10^5	1.34×10^3	4.92×10^5	8.98×10^5
pentacosane	7.94×10^5	1.28×10^3	< DL	7.94×10^5
hexacosane	5.58×10^5	1.06×10^3	< DL	5.58×10^5
heptacosane	3.96×10^5	9.53×10^2	< DL	3.96×10^5
octacosane	2.48×10^5	< DL	< DL	2.48×10^5
nonacosane	2.01×10^5	< DL	< DL	2.01×10^5

<DL = below detection limits of analytical method

Table A.5 Alkane concentrations in sediment samples from Fishing Ground

	Concentration (μg/kg)		
	Fishing Ground (Top)	Fishing Ground (Middle)	Fishing Ground (Bottom)
Undecane	< DL	< DL	< DL
dodecane	3.90×10^5	$4.30\ 10^5$	< DL
tridecane	4.75×10^5	8.34×10^5	2.13×10^5
tetradecane	6.90×10^5	8.86×10^5	3.98×10^5
pentadecane	2.15×10^6	1.28×10^6	8.67×10^5
hexadecane	1.90×10^6	1.22×10^6	8.37×10^5
heptadecane	1.24×10^6	9.32×10^5	5.81×10^5
octadecane	6.23×10^5	7.21×10^5	3.73×10^5
nonadecane	3.19×10^5	4.58×10^5	2.05×10^5
eicosane	4.34×10^5	5.47×10^5	2.80×10^5
heneicosane	5.05×10^5	5.41×10^5	3.51×10^5
docosane	3.26×10^5	3.22×10^5	2.00×10^5
tricosane	4.08×10^5	3.97×10^5	3.02×10^5
tetracosane	< DL	4.17×10^5	< DL
pentacosane	< DL	< DL	< DL
hexacosane	< DL	< DL	< DL
heptacosane	< DL	< DL	< DL
octacosane	< DL	< DL	< DL
nonacosane	< DL	< DL	< DL

<DL = below detection limits of analytical method

Table A.6 Major and trace ion concentrations in water samples from October 2014 field trip. Standard deviation of duplicate samples is included.

	Concentration (mg/kg)		
	PBFG	PBSP	GB
B^{3+}	2787.942 (± 504.461)	3000.666 (± 250.373)	3275.121 (± 206.517)
Li^{+}	104.093 (± 17.336)	113.545 (± 5.982)	120.611 (± 10.549)
Be^{2+}	<DL	<DL	<DL
$Si^{2+,4+,4-}$	<DL	<DL	<DL
Al^{3+}	17.208 (± 6.719)	32.754 (± 5.737)	21.287 (± 5.618)
P^{3-}	<DL	<DL	<DL
Ca^{2+}	301975 (± 55016)	325771 (± 36031)	353980 (± 26067)
$Ni^{2+,3+}$	<DL	<DL	<DL
As^{3-}	<DL	<DL	<DL
Se^{4+}	<DL	<DL	<DL
Rb^{+}	87.006 (± 13.125)	94.488 (± 10.318)	100.798 (± 8.238)
Sr^{2+}	4996.884 (± 817.348)	5381.982 (± 648.973)	5807.919 (± 477.732)
Mo^{6+}	7.940 (± 1.182)	9.356 (± 0.534)	9.115 (± 0.540)
Ag^{+}	<DL	0.504 (± 0.338)	0.156*
Hg^{2+}	<DL	0.517*	<DL
$Tl^{+,3+}$	<DL	<DL	<DL
Pb^{2+}	0.465 (± 0.072)	0.095 (± 0.017)	0.129 (± 0.015)
Bi^{3+}	<DL	<DL	<DL
S^{2-}	719161 (± 129041)	777666 (± 84196)	850561 (± 67118)
Mg^{2+}	754022 (± 134791)	813494 (± 91919)	890289 (± 69722)
$V^{3+,5+}$	<DL	<DL	<DL
$Fe^{2+,3+}$	972.554 (± 132.014)	1169.191 (± 174.534)	1150.761 (± 80.380)
$Mn^{2+,4+}$	2.208 (± 0.452)	10.233 (± 0.749)	16.341 (± 0.986)
$Co^{2+,3+}$	<DL	<DL	<DL
Zn^{2+}	29.782 (± 4.292)	25.038 (± 15.061)	12.215 (± 0.306)
$Cu^{+,2+}$	55.221 (± 6.503)	50.525 (± 19.271)	61.608 (± 5.972)
Cd^{2+}	<DL	<DL	<DL

<DL = below detection limits of analytical method, * = number is not an average.

Table A.7 Major and trace ion concentrations in sediment samples. Standard deviation of duplicate samples is included.

	Concentration (mg/kg)		
	PBFG (top)*	PBSP (top)	GB (top)
B^{3+}	61.32	56.40 (± 2.00)	64.18 (± 0.26)
Li^{+}	325.15	56.17 (± 14.63)	79.21 (± 13.63)
Be^{2+}	<DL	<DL	<DL
Si^{4+}	8.91	11.99 (± 2.49)	40.51 (± 9.08)
Al^{3+}	41080.43	37045.94 (±26.15)	59682.61 (± 1051.87)
P^{3-}	877.46	502.48 (± 8.76)	483.39 (± 16.79)
Ca^{2+}	10173.23	19403.33 (± 219.77)	36137.93 (± 709.45)
$Ni^{2+,3+}$	17.82	31.39 (± 0.97)	30.25 (± 1.83)
As^{3-}	17.82	16.84 (± 2.11)	25.64 (± 5.09)
Se^{4+}	<DL	<DL	<DL
Rb^{+}	530.04	-	505.57 (± 128.24)
Sr^{2+}	80.17	87.02(± 0.06)	266.82 (± 2.86)
Mo^{6+}	<DL	<DL	<DL
Ag^{+}	<DL	<DL	<DL
Hg^{2+}	<DL	<DL	<DL
$Tl^{+,3+}$	<DL	<DL	<DL
Pb^{2+}	<DL	<DL	<DL
Bi^{3+}	<DL	<DL	<DL
Mg^{2+}	14297.75	18485.28 (± 267.29)	12129.94 (± 218.70)
$V^{3+,5+}$	84.63	53.34 (± 4.15)	127.90 (± 2.42)
$Fe^{2+,3+}$	19045.86	24039.12 (±364.95)	40269.55 (± 1319.65)
$Mn^{2+,4+}$	195.98	1116.50 (± 8.83)	905.86 (± 47.23)
$Co^{2+,3+}$	-	94.17 (± 2.93)	43.57 (± 1.79)
Zn^{2+}	22.27	11.99 (± 2.49)	8.46 (± 2.72)
$Cu^{+,2+}$	17.82	29.10 (± 2.26)	10.77 (± 1.09)
Cd^{2+}	<DL	<DL	<DL

- = unable to integrate due to bad peaks, * = sample does not represent an average

<DL = analyte below detection limits of analytical methods.

Table A.8 Trace metal concentrations in crude oil sample from Shoal Point. Standard deviation of triplicate samples is included.

	Concentration (mg/kg)		Concentration (mg/kg)
B^{3+}	1575.86 (± 12.52)	Hg^{2+}	<DL
Li^{+}	30.29 (± 8.05)	$Tl^{+,3+}$	<DL
Be^{2+}	<DL	Pb^{2+}	<DL
Si^{4+}	35.59 (± 0.58)	Bi^{3+}	<DL
Al^{3+}	<DL	S^{2-}	483280.23 (± 7829.77)
P^{3-}	34.23 (± 6.01)	Mg^{2+}	123321.80 (± 603.75)
Ca^{2+}	41413.60 (± 110.13)	$V^{3+,5+}$	<DL
$Ni^{2+,3+}$	<DL	$Fe^{2+,3+}$	<DL
As^{3-}	<DL	$Mn^{2+,4+}$	<DL
Se^{4+}	<DL	$Co^{2+,3+}$	<DL
Rb^{+}	<DL	Zn^{2+}	<DL
Sr^{2+}	909.13 (± 4.57)	$Cu^{+,2+}$	13.13*
Mo^{6+}	<DL	Cd^{2+}	<DL
Ag^{+}	<DL		

<DL signifies analyte was below detection limits of analytical method (0.01 mg/kg)

* = signifies this sample does not represent an average.

Table A.9 Major and trace metal concentrations in mussels. Standard deviation of duplicate samples is included.

	Concentration (mg/kg)				
	GB	PBFW		GB	PBFW
B^{3+}	61.84 (± 9.00)	65.06 (± 7.62)	Hg^{2+}	<DL	<DL
Li^{+}	<DL	<DL	$Tl^{+,3+}$	<DL	<DL
Be^{2+}	<DL	<DL	Pb^{2+}	-	-
Si^{4+}	8328.50 (±11678.29)	43202.86 (± 7535.02)	Bi^{3+}	-	-
Al^{3+}	139.49 (± 143.97)	1217.62 (± 721.95)	Mg^{2+}	6660.23 (± 1396.16)	6100.87 (± 4467.85)
P^{3-}	-	-	$V^{3+,5+}$	<DL	<DL
Ca^{2+}	4891.18 (± 3292.11)	7292.19 (± 6044.97)	$Fe^{2+,3+}$	196.85 (± 107.41)	736.41 (± 438.24)
$Ni^{2+,3+}$	7.87 (± 2.80)	8.02 (± 0.90)	$Mn^{2+,4+}$	5.60 (± 2.96)	20.75 (± 3.03)
As^{3-}	16.41 (± 3.29)	18.88 (± 1.20)	$Co^{2+,3+}$	<DL	<DL
Se^{4+}	16.07 (± 5.15)	16.38 (± 2.95)	Cd^{2+}	<DL	<DL
Rb^{+}	<DL	<DL	$Cu^{+,2+}$	7.13 (± 2.17)	8.24 (± 1.11)
Sr^{2+}	70.88 (±49.21)	70.41 (± 22.61)	Zn^{2+}	54.00 (± 32.51)	119.15 (± 5.65)
Mo^{6+}	10.00 (± 2.16)	11.04 (± 0.70)	Ag^{+}	4.52 (± 0.80)	4.53 (± 0.49)

- = unable to integrate due to bad peaks

<DL = analyte was below detection limits of analytical methods

Table A.10 Mussel Health Indices

St. George's Bay			Port au Port Bay		
Length (cm)	Width (cm)	Wet Weight (cm)	Length (cm)	Width (cm)	Wet Weight (cm)
68.63	34.24	47.0806	62.28	31.58	25.9414
78.88	36.23	89.0635	61.3	33.34	34.6156
66.17	37.18	41.8874	66.29	31.54	40
61.79	31.58	23.966	60.56	27.23	33.8594
67.45	33.48	51.4605	56.23	31.46	26.0705
54.25	29.06	20.8594	61.42	25.49	29.739
84.55	45.26	99.2154	63.24	30.06	28.9745
			73.68	32.59	47.8715
			66.11	34	30.7655
			71.91	36.85	38.636
			71.63	34.84	33.227
			72.46	36.17	41.7306
			84.41	35.57	51.783
			70.5	32.33	41.0929
			76.02	38.83	53.0938
			72.23	34.19	35.28
			61.59	32.1	28.5253
			74.2	37.69	50.1017
			75.3	38.06	34.6902
			65.47	30.93	29.5306
			72.61	34.28	35.1451
			73.87	37.27	50.1149
			77.08	36.49	38.3056
			59.24	28.17	25.7228
			69.3	34.68	36.8383
			72.39	37.32	57.992

Table A.10 continued on next page

Table A.10 (continued)

St. George's Bay			Port au Port Bay		
Length (cm)	Width (cm)	Wet Weight (cm)	Length (cm)	Width (cm)	Wet Weight (cm)
			70.93	32.43	35.0745
			65.93	33.37	38.815
			75.95	30.4	46.0108
			69.93	33.66	38.3986
			71.6	37.28	55.0233
			74.58	32.52	39.4881
			74.32	36.64	41.5231
			73.57	36.71	53.9868
			60.33	30.89	31.9723
			69.91	30.86	40.6962
			62.53	30.01	37.3037
			66.99	34.94	39.8295
			58.96	27.66	28.2821
			65.72	34.41	31.6804
			73.27	34.45	36.6752
			72.94	32.86	43.7164
			69.24	32.55	42.7846
			69.07	33.73	32.0912
			70.21	33.35	49.9858
			66.56	33.42	36.6735
			72.19	34.14	38.5349
			67.08	32.85	44.5839
			63.08	30.35	37.5536

References

(CCME), C.C.o.M.o.t.E., 2008, Canadian Environmental Protection Act (CEPA). Interim Sediment Quality Guidelines.

Arora, A., 2006, Hydrocarbons (Alkanes, Alkenes And Alkynes), Discovery Publishing House Pvt. Limited.

Atlas, R.M., and Hazen, T.C., 2011, Oil Biodegradation and Bioremediation: A Tale of the Two Worst Spills in U.S. History: Environmental Science & Technology, v. 45, p. 6709-6715.

Barron, M.G., 2012, Ecological Impacts of the Deepwater Horizon Oil Spill: Implications for Immunotoxicity: Toxicologic Pathology, v. 40, p. 315-320.

Beer, T., 1996, Environmental oceanography: Boca Raton, Boca Raton : CRC Press.

Blummer, M., Souza, G., and Sass, J., 1970, Hydrocarbon pollution of edible shellfish by an oil spill: Marine Biology, v. 5, p. 195-202.

Bradl, H.B., 2005, Heavy Metals in the Environment: Origin, Interaction and Remediation: Netherlands, Elsevier.

Burns, K.A., and Smith, J.L., 1981, Biological monitoring of ambient water quality: the case for using bivalves as sentinel organisms for monitoring petroleum pollutants in coastal waters: Estuarine, Coastal and Self Science, v. 13, p. 433-443.

Canada, E., 1994, Polycyclic Aromatic Hydrocarbons, *in* Canada, H., ed.

Canada, E., 2013, Mercury in the Food Chain, *in* Change, E.a.C., ed.

Carls, M.G., Babcock, M.M., Harris, P.M., Irvine, G.V., Cusick, J.A., and Rice, S.D., 2001, Persistence of oiling in mussel beds after the Exxon Valdez oil spill: Marine Environmental Research, v. 51, p. 167-190.

CBC, 2012, Pacific Ocean acid levels jeopardizing marine life CBC news: Vancouver, CBC.

CBC, 2015, 'Nothing alive' in Port au Port Bay because of oil spill, fisherman says, CBC news.

CCME, 1996, Canadian Water Quality Guidelines for the Protection of Aquatic Life: Dissolved Oxygen (Marine), *in* Environment, C.C.o.M.o.t., ed.

DFO, 2016, European Green Crab, *in* Canada, G.o., ed.

Downs, C.A., Shigenaka, G., Fauth, J.E., Robinson, C.E., and Huang, A., 2002, Cellular Physiological Assessment of Bivalves after Chronic Exposure to Spilled Exxon Valdez Crude Oil Using a Novel Molecular Diagnostic Biotechnology: Environmental Science & Technology, v. 36, p. 2987-2993.

EPA, 2000, Ambient Aquatic Life Water Quality Criteria for Dissolved Oxygen (Saltwater): Cape Cod to Cape Hatteras. , *in* Technology, O.o.W.O.o.S.a., ed.: Washington, DC.

EPA, U., 1995, Light Nonaquaeous Liquids, *in* Issue, G.W., ed.

Fahim, M.A., Al-Sahhaf, T.A., and Elkilani, A.S., 2010, Fundamentals of Petroleum Refining Kidlington, Oxford, Elsevier, 496 p.

Faure, G., 1986, Principles of isotope geology: New York, New York : Wiley.

Gale, F., 2014, Pollutants blamed for Port au Port Bay scallop fishery collapse, Western Star: Corner Brook.

Garcia, E.G., 2006, The Fishery for Iceland Scallop (Chlamys islandica) in the Northeast Atlantic, Advances in Marine Biology, Volume Volume 51, Academic Press, p. 1-55.

Geraldine McCormick-Ray, M., 1987, Hemocytes of Mytilus edulis affected by Prudhoe Bay crude oil emulsion: Marine Environmental Research, v. 22, p. 107-122.

Hadfield, M., and Wang, Y., 2003, Tribology in Environmental Design 2003, John Wiley & Sons.

Hannam, M.L., Bamber, S.D., Galloway, T.S., Moody, A.J., and Jones, M.B., 2010a, Effects of the model PAH phenanthrene on immune function and oxidative stress in the haemolymph of the temperate scallop Pectin maximus: Chemosphere, v. 78, p. 779-784.

Hannam, M.L., Bamber, S.D., Moody, A.J., Galloway, T.S., and Jones, M.B., 2010b, Immunotoxicity and oxidative stress in the Artic scallop Chlamy islandica: Effects of acute oil exposure: Ecotoxicology and Environmental Saftey, v. 73, p. 1440-1448.

Hicks, L., and Owens, J., The History of Petroleum Exploration in Western Newfoundland, *in* Department of Natural Resources, E.B., ed.

Hicks, L., and Owens, J., 2014, The History of Petroleum Exploration in Western Newfoundland, *in* Department of Natural Resources, E.B., ed.

Hillier, B., 2014, Shellfish Mystery in Port au Port Bay, West Coast Morning Show: Cornerbrook, Newfoundland, Canada.

Hinchey, A., Knight, I., Kilfoil, G., Hynes, K., Middleton, D., Hicks, L., and 2015, The Green Point Shale of Western Newfoundland, *in* Resources, D.o.N., ed.: A Review of its Geological Setting, its Potential as

as Unconventional Hydrocarbon Reservior, and its Ability to be Safely Stimulated Using the

Technique of Hydraulic Fracturing, Government of Newfoundland and Labrador

Hitchman, M.L., 1978, Measurement of dissolved oxygen: New York, New York : Wiley.

Hume, M., 2014, Mystery surrounds massive die-off of oysters and scallops off B.C. coast, The Globe and Mail: Vancouver

Iwegbue, C.M.A., Aganbi, E., Obi, G., Osakwe, S.A., Eguvbe, P.M., Ogala, J.E., and Martincigh, B.S., 2016, Aliphatic hydrocarbon profiles in sediments of the Forcados River, Niger Delta, Nigeria: Environmental Forensics, v. 17, p. 144-155.

Jonasson, J.P., Thorarinsdottir, G., Eiriksson, H., Solmundsson, J., and Marteinsdottir, G., 2007, Collapse of the fishery for Iceland scallop (Chlamys islandica) in Breidafjordur, West Iceland: ICES Journal of Marine Science: Journal du Conseil, v. 64, p. 298-308.

Kester, D.R., Duedall, I.W., Connors, D.N., and Pytkowicz, R.M., 1967, PREPARATION OF ARTIFICIAL SEAWATER1: Limnology and Oceanography, v. 12, p. 176-179.

Kjeldsen, P., Barlaz, M.A., Rooker, A., Baun, A., Ledin, T., and Christensen, P., 2002, Present and Long-Term Composition of MSW Landfill Leachate: A Review, *in* Kjeldsen, P., ed., Volume 32.

Luckey, T.D., Venugopal, B., and Hutcheson, D., 1975, Heavy Metal Toxicity, Safety and Hormology: New York, Academic Press.

Mahmoudi, N., Porter, T.M., Zimmerman, A.R., Fulthorpe, R.R., Kasozi, G.N., Silliman, B.R., and Slater, G.F., 2013, Rapid Degradation of Deepwater Horizon Spilled

Oil by Indigenous Microbial Communities in Louisiana Saltmarsh Sediments: Environmental Science & Technology, v. 47, p. 13303-13312.

Mohammad, Z., Bahram, H.K., and Reza, S.M., 2015, Allometry, condition index and secondary production in bivalve Barbatia decussata on rocky intertidal shores in the Northern Persian Gulf, Iran: Journal of Environmental Biology, v. 36, p. 1185-1192.

Morrill, P.L., Szponar, N., Johnston, M., Marvin, C., and Slater, G.F., 2014, Deciphering microbial carbon substrates in PAH contaminated sediments using phospholipid fatty acids, and compound specific δ13C and Δ14C: Organic Geochemistry, v. 69, p. 76-87.

O'Gorman, B., 2014, *in* Cook, M., ed.: West Bay.

Osuji, L.C., and Onojake, C.M., 2004, Trace Heavy Metals Associated with Crude Oil: A Case Study of Ebocha-8 Oil-Spill-Polluted Site in Niger Delta, Nigeria: Chemistry & Biodiversity, v. 1, p. 1708-1715.

Pampanin, D.M., and Sydnes, M.O., 2013, Polycyclic Aromatic Hydrocarbons a Constituent of Petroleum: Presence and Influence in the Aquatic Environment, Hydrocarbon: Rijeka, InTech, p. Ch. 0.

Petsch, S.T., Eglinton, T.I., and Edwards, K.J., 2001, ^{14}C-Dead living biomass: Evidence for microbial assimilation of ancient organic carbon during shale weathering: Science, v. 292, p. 1127-1131.

Reddy, C.M., Xu, L., Eglinton, T.I., Boon, J.P., and Faulkner, D.J., 2002, Radiocarbon content of synthetic and natural semi-volatile halogenated organic compounds: Environmental Pollution, v. 120, p. 163-168.

Samuels, L., Kunst, L., and Jetter, R., 2008, Sealing Plant Surfaces: Cuticular Wax Formation by Epidermal Cells: Annual Review of Plant Biology, v. 59, p. 683-707.

Schirmer, A., Rude, M.A., Li, X., Popova, E., and del Cardayre, S.B., 2010, Microbial Biosynthesis of Alkanes: Science, v. 329, p. 559-562.

Sharp, Z., 2007, Principles of stable isotope geochemistry: Upper Saddle River, N.J., Upper Saddle River, N.J. : Pearson/Prentice Hall.

Shiel, A.E., Weis, D., and Orians, K.J., 2012, Tracing cadmium, zinc and lead sources in bivalves from the coasts of western Canada and the USA using isotopes: Geochimica Et Cosmochimica Acta, v. 76, p. 175-190.

Smith, J.N., Lee, K., Gobeil, C., and Macdonald, R.W., 2009, Natural rates of sediment containment of PAH, PCB and metal inventories in Sydney Harbour, Nova Scotia: Science of the Total Environment, v. 407, p. 4858-4869.

Taylor, R.E., and Bar-Yosef, O., 2014, Radiocarbon Dating, Second Edition : An Archaeological Perspective (2): Albuquerque, US, Left Coast Press.

Tillman, J.A., Seybold, S.J., Jurenka, R.A., and Blomquist, G.J., 1999, Insect pheromones—an overview of biosynthesis and endocrine regulation: Insect Biochemistry and Molecular Biology, v. 29, p. 481-514.

Wang, L.K., Chen, J.P., Hung, Y.-T., and Shammas, N.K., 2009, Heavy Metals in the Environment, Taylor & Francis Group.

Wardle, R.J., 2000, Mineral Commodities of Newfoundland and Labradour Zinc and Lead, *in* Newfoundland, G.S.o., ed.

Wiens, J.A., 2013, Oil in the Environment Legacies and Lessons of the Exxon Valdez Oil Spill: New York, Cambridge University Press.

Yunker, M.B., Macdonald, R.W., Vingarzan, R., Mitchell, R.H., Goyette, D., and Sylvestre, S., 2002, PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition: Organic Geochemistry, v. 33, p. 489-515.